

ARCHIVES OF PATHOLOGY

EDITORIAL BOARD

LUDVIG HEKTOEN, Chicago, Chief Editor

GRANVILLE A. BENNETT, Chicago

WILEY DAVIS FORBUS, Durham, N. C.

S. B. WOLBACH, Boston

GEORGE H. WHIPPLE, Rochester, N. Y.

FRANK R. MENNE, Portland, Ore.

Volume 46
1948

PUBLISHERS
AMERICAN MEDICAL ASSOCIATION
CHICAGO, ILL.

CONTENTS OF VOLUME 46

JULY 1948. NUMBER 1

	PAGE
Genesis of the Gangrenous and Reparative Processes in Trench Foot. Matthew Block, M.D., Ph.D., Chicago.....	1
Development of a State Refractory to Growth of a Mouse Tumor Implanted in the Anterior Chamber of the Eye of the Guinea Pig. John A. Schilling, M.D., and Albert C. Snell Jr., M.D., Rochester, N. Y.....	35
Paradoxical Embolism: A Review of the Literature, with Report of a Case in Which This Condition Followed the Administration of "Dicumarol." Raymond L. Young, M.D.; R. C. Derbyshire, M.D., and O. S. Cramer, M.D., Albuquerque, New Mexico.....	43
Hereditary Renal Disease and Amyloidosis in Mice. W. E. Heston, Ph.D., and Margaret K. Deringer, Ph.D., Bethesda, Md.....	49
Visceral Lesions in a Case of Rheumatoid Arthritis. Peter Gruenwald, M.D., New York.....	59
Case Reports:	
Ewing's Endothelial Myeloma of Adolescents: Report of Two Fatal Cases. Horace K. Giffen, M.D., Youngstown, Ohio.....	68
Dyschondroplasia with Hemangiomatosis (Maffucci's Syndrome) and Teratoid Tumor of the Ovary. J. F. Kuznia, M.D., and J. M. King, M.D., Milwaukee.....	74
Laboratory Methods and Technical Notes:	
Argentaffin Cells of the Human Appendix: A Comparative Study of the Results Obtained with Modified Schmorl and Masson Technics. A. Laskey, B.S., and J. Greco, B.S., Bethesda, Md.....	83
Books Received.....	86

AUGUST 1948. NUMBER 2

Diffuse Angiectasis of the Cerebral Meninges of the Newborn Infant: Report of Three Cases. Edith L. Potter, M.D., Ph.D., Chicago.....	87
Specific Gravity of the Blood Corpuscle: Its Possible Significance in Atherosclerosis: Israel Gordon, M.D., M.R.C.P., D.P.H., Ilford, Essex, England	97
Influence of Local Acidification of Tissue Bordering Cancerous Growths, with Special Reference to the Eosinophil, the Paneth Cell and the Acidophilic Plasma Cell. Charles E. Black, M.D., and R. Sorensen Ogle, B.S., Lansing, Mich.....	107

AUGUST—*Continued*

	PAGE
Postmortem Examination of Teeth and Supporting Structures to Aid in Personal Identification. Myron J. Van Leeuwen, D.D.S., Boston.....	119
Control of Hepatic Coccidiosis of Rabbits with Succinylsulfathiazole U.S.P.: A Study of the Mode of Action of the Sulfonamides. Michele Gerundo, M.D., Ph.D., Honolulu, Territory of Hawaii.....	128
Significance of Agonal Changes in the Human Liver. Hans Popper, M.D., Ph.D., Chicago	132
Mechanisms of Leukopenia with Inflammation: An Additional Leukopenic Factor Found in Alkaline Exudates. Valy Menkin, M.D., Philadelphia...	145
Significance of the Beta Granules in the Islets of Langerhans of the Pancreas. S. S. Barron, M.D., Minneapolis.....	159
Nucleic Acids and Cytologic Changes in Regenerating Rat Liver. Robert E. Stowell, M.D., Ph.D., St. Louis.....	164
Mitotic Activity in the Aortic Lesions of Experimental Cholesterol Atherosclerosis of Rabbits. Gardner C. McMillan, M.D., Ph.D., and G. Lyman Duff, M.D., Ph.D., Montreal, Canada.....	179
Case Reports:	
Diffuse Plasma Cell Myelomatosis. E. Stark, M.D., and E. L. Amidon, M.D., Burlington, Vt.....	183
Adenoma of the Parotid Gland. John T. Godwin, M.D., New York, and S. H. Colvin Jr., M.D., New Orleans.....	187
Books Received.....	190

SEPTEMBER 1948. NUMBER 3

Intracranial Vascular Lesions in Late Rheumatic Heart Disease. John Denst, M.D., and Karl T. Neuburger, M.D., Denver.....	191
Myocardial Changes in Poliomyelitis. Vera B. Dolgopoul, M.D., and Mary D. Cragan, M.D., New York.....	202
Comparison of Thymic Hyperplasia in Myasthenia Gravis and Exophthalmic Goiter. Allen L. Bryan, M.D.; John R. McDonald, M.D., and O. Theron Clagett, M.D., Rochester, Minn.....	212
Testicular Tumors: II. Interstitial Cell and Miscellaneous Neoplasms. Robert E. Scully, M.D., Walpole, Mass., and Asa R. Parham, M.D., Boston.....	229
Some Problems Related to the Origin and Meaning of Pituitary Gland Tumors. I. Costero, M.D., Mexico City.....	243
Effect of Sodium Chloride Deprivation on the Growing Rat. John T. Cuttino, M. D.; A. S. Paris, M.D., and Macey H. Rosenthal, M.D., Durham, N. C..	260
General Reviews:	
Interrelationship of Diseases of the Liver and the Brain. A. B. Baker, M.D., Minneapolis	268
Notes and News.....	287
Books Received.....	288

OCTOBER 1948. NUMBER 4

	PAGE
Sarcoidosis Involving the Heart: Report of Case with Sudden Death. Thomas M. Scotti, M.D., and Charles E. McKeown, M.D., Richmond, Va.....	289
Chronic Inflammatory Lesions of Skeletal Muscle in Rheumatoid Arthritis and in Other Diseases. M. A. Ogryzlo, Toronto, Canada.....	301
Plasma Cell Mastitis. Béla Halpert, M.D.; Joe M. Parker, M.D., and Joseph M. Thuringer, M.D., Oklahoma City.....	313
A Quantitative Approach to the Study of Splenomegaly. Alvin J. Gordon, M.D.; Ernest C. Holder, M.D., and Sergei Feitelberg, M.D., New York..	320
Viral Versus Toxic Hepatic Necrosis. Hans Popper, M.D., Ph.D., and Murray Franklin, M.D., Chicago.....	338
Hepatic Heterotopy in the Splenic Capsule. George J. Heid Jr., M.D., and Emmerich Von Haam, M.D., Columbus, Ohio.....	377
Medionecrosis of the Aorta. George D. Amromin, M.D.; Jakub G. Schlichter, M.D., and A. J. L. Solway, M.D., Chicago.....	380
Mammary Lipoma. C. G. Tedeschi, M.D., Framingham, Mass.....	386
Absence of Renal Lesions in Rats Receiving a Synthetic Diet Low in Protein. Elizabeth Lowenhaupt, M.D., San Francisco.....	398
Books Received.....	400

NOVEMBER 1948. NUMBER 5

Aging Processes in the Ovaries of Mice Belonging to Strains Differing in the Incidence of Mammary Carcinoma. Leo Loeb, M.D., St. Louis.....	401
Effects of Folic Acid Antagonists Inoculated in Embryonated Eggs. Philip F. Wagley, M.D., and Herbert R. Morgan, M.D., Boston.....	441
Cholesterol of the Human Adrenal Gland: Its Significance in Relation to Adrenal Function and Structure. Walter F. Rogers Jr., M.D., Syracuse, N. Y., and Robert H. Williams, M.D., Seattle.....	451
Reticulum Cell Sarcoma of Bone. V. R. Khanolkar, Bombay, India.....	467
Neoplastic Diseases of Dogs: II. Mast Cell Sarcoma, Lymphosarcoma, Histiocytoma. R. M. Mulligan, M.D., Denver.....	477
Case Reports:	
Fatal Viral Hepatitis Complicated by Phlegmonous Cecitis and Ileocecal Intussusception. Commander William Umiker (MC), U.S.N., Bethesda, Md.....	493
Laboratory Methods and Technical Notes:	
Method for Biopsy of Bone Marrow of Experimental Animals. H. B. Ritter, B.A., and J. J. Oleson, Ph.D., Pearl River, N. Y.....	498
Notes and News.....	501
Books Received.....	502

	PAGE
Cytologic Changes in Bronchogenic Carcinoma Following Treatment with Nitrogen Mustard (Methyl-Bis [β -Chloroethyl] Amine). Edward A. Gaensler, M.D.; Donald G. McKay, M.D.; Paul F. Ware, M.D., and Joseph P. Lynch, M.D., Boston.....	503
Effect of Nitrogen Mustard in Mycosis Fungoides. Matthew Block, Ph.D., M.D., and John C. Murphy, M.D., Chicago.....	519
Bronchial Adenoma Producing an "Alveolar Cell Carcinoma" Pattern. J. H. Cheek, M.D., and E. E. Muirhead, M.D., Dallas, Texas.....	529
Experimental Atherosclerosis: X. The Effect of Desoxycorticosterone Acetate on the Cholesterol Content of the Blood, the Aorta and the Liver of the Rabbit. Maurice Bruger, M.D., and Bertrand E. Lowenstein, M.D., New York.....	536
Silvering of Lepra Bacilli in Tissues. F. León Blanco, M.D., Habana, Cuba, and G. L. Fite, M.D., Carville, La.....	542
Splenic Cysts. H. T. Tamaki, M.D., Norristown, Pa.....	550
Effects of Folic Acid Deficiency and a Folic Acid Antagonist on Chicks. Ephraim Woll, M.D., Burlington, Vt.....	559
General Reviews:	
Spontaneous Demyelinating Diseases of Animals: A Study in Comparative Pathology. Lester S. King, M.D., and Marjorie C. Meehan, M.D., Chicago.....	567
Notes and News.....	599
General Index.....	601

GENESIS OF THE GANGRENOUS AND REPARATIVE PROCESSES IN TRENCH FOOT

MATTHEW BLOCK, M.D., Ph.D.*
CHICAGO

THIS REPORT is part of a study of 19 cases of trench foot seen at Camp Butner General Hospital in the early months of 1945. Biopsies were done in 15 of the cases, and the results were presented in another report.¹ It was impossible to differentiate by any morphologic criteria between the tissues from the 15 patients with trench foot and those from 8 normal healthy controls. Except for the history of having been exposed to cold wet weather, there was no objective means of separating the men without any loss of tissue from other hospital patients with no history of cold injury.² Ten of the 19 men suffered injury severe enough to have lost some tissue, the loss varying from a part of a single toe to both feet. This report deals specifically with the pathologic aspects of these amputated specimens.

CLINICAL HISTORY

The clinical history of trench foot and related diseases has been amply presented in the recent and even in the ancient literature.³ The

*Formerly Captain, Medical Corps, Army of the United States; now Senior Research Fellow, United States Public Health Service, Department of Medicine, University of Chicago.

1. Block, M.: Arch. Path. 44:360, 1947.
2. (a) Krause, L.: Personal communication to the author. (b) Silverman, J.: Ann. Int. Med. 22:702, 1946. (c) Block.¹
3. (a) Larrey, D. J.: Mémoires de chirurgie militaire et campagnes, Paris, C. S. Smith, 1812. (b) Ungley, C. C., and Blackwood, W.: Lancet 2:447, 1942. (c) Lesser, A.: Ann. Surg. 21:257, 1945. (d) Patterson, R. H., and Anderson, F. M.: Surg., Gynec. & Obst. 80:1, 1945. (e) Friedman, N.: Am. J. Path. 21:387, 1945; (f) Am. J. Clin. Path. 16:634, 1946. (g) Webster, D. R.; Woolhouse, F. M., and Johnston, J. L.: J. Bone & Joint Surg. 24:185, 1942. (h) White, J. C.: New England J. Med. 228:211 and (i) 241, 1943. (j) White, J. C., and Scoville, W.: ibid. 232:415, 1944. (k) White, J. C., and Warren, S.: War Med. 5:613, 1944. (l) Wieting: Zentralbl. f. Chir. 40:593, 1913. (m) Wright, I. S., and Allen, E. V.: Bull. U. S. Army M. Dept., 1943, no. 65, p. 136. (n) Leriche, R., and Kunlin, J.: Progrès méd. 68:167, 1940.

first accurate account was written by Larrey^{3a} on the basis of what he experienced during Napoleon's retreat from Moscow. In modern times, Wieting³¹ gave a clear description of the disease as it occurred in the Balkan Wars.

In the present report the typical case was that of an infantryman of the 19 to 25 year age group, in previous good health, with the rank of private or corporal, who participated in the campaign of the American Armies in France and Germany in the winter of 1944-1945. His history is that of a patient who underwent a severe enough exposure to have suffered marked loss of tissue.

He arrived in France on Dec. 15, 1944 as a member of the 70th Infantry Division. He went into combat on January 2, 1945, and from that time on, his feet were continually cold and wet. The weather alternated between snow and rain, with days during which the ground was frozen solid for a few hours. The patient was wearing well fitting combat shoes over socks that were half wool and half cotton. He was usually able to alternate his socks during the first few days, drying his reserve pair against his body. Because his shoes were wet, his socks became wet within a few moments of changing. He never wore "shoepacs," because other men said they caused the feet to sweat profusely. He did not wear galoshes. During the last three days of combat he was unable to take time to remove his shoes.

About January 5, he noted that his feet were pale, swollen, painful, and gave a sensation of burning, and that numbness was developing. The next day he was able to get into his shoes only with great difficulty.

His company attacked on January 6, and he walked through the melting snow for twenty-four hours. On January 7 he was on outpost duty, lying in his foxhole, with his feet hanging down in the melted snow. The next morning his feet were blue and cold and much more painful. They burned and tingled, and he felt as though he were walking on stumps. By January 9, he was hardly able to walk, and when he turned in at the battalion aide station, his shoes had to be cut off because of the swelling of his feet.

The same day he was evacuated from his company, and on January 10, while he was on the way to a general hospital, blue-red, fluid-filled blisters began to appear on his toes. Beginning with January 9, his feet were kept at room temperature and thereafter became much more inflamed and painful. On January 12 gangrene developed in his toes, appearing first in the big toe of each foot as a black area under the nail and then affecting all the other toes. By January 16, the gangrenous skin reached the midtarsal areas, and the heels were covered with heavy dark hyperkeratotic skin, with some fairly normal tissue between toe and heel. The front halves of his feet were black and dried out like old shoe leather.

The black skin began to exfoliate about the end of January, leaving the skin on his heels a delicate pink, as after a mild burn. The peeling ceased at the ends of the metatarsal bones and a demarcation zone became evident, while the toes became even more dried out and contractures began at the phalangeal joints. By March he was able to walk, regardless of the gangrenous toes, which were but slightly disabling in spite of the extensiveness and severity of the gangrene.

He had been given injections of both penicillin and tetanus antitoxin. His feet were kept uncovered because when covered by blankets they were painful. On April 17 guillotine amputation was done bilaterally through the distal ends of the meta-

tarsal bones, and on June 6 dermatome skin grafts were applied to the granulating surface of his feet.

The site of amputation broke down about three months later when his feet were trampled. In November of 1946 his feet and hands perspired profusely, the distal ends of his feet were blue-red, the skin was atrophic, the deeper tissue was hard and fibrous, and when his feet were hanging they became abnormally cyanotic and edematous. The dorsalis pedis and posterior tibial pulses were palpable. He could not keep his feet under blankets at night, and they were susceptible to extremes of weather.

This history was duplicated in thousands of other cases, the only variable being the intensity of exposure as it influenced the resultant injury. On the other hand, there was always a variation in the extent of injury in men exposed to the same environment. There can be little doubt that there was a marked individual difference in the quantitative reaction to cold. Some men had less severe injury, and within a few weeks after evacuation the gangrenous skin exfoliated, leaving a healthy pink covering on their feet. In others, with more severe injury, the whole of both feet were lost. Breakdown of tissue rarely occurred after amputation. Usually the feet gradually assumed a much more normal appearance, and the men were eventually able to keep their feet under blankets.

MATERIALS AND METHODS

The tissues examined were all obtained at amputation prior to the onset of any secondary infection. A demarcation zone had set in, and the mummified tissue had already begun to contract like an old paper bag.

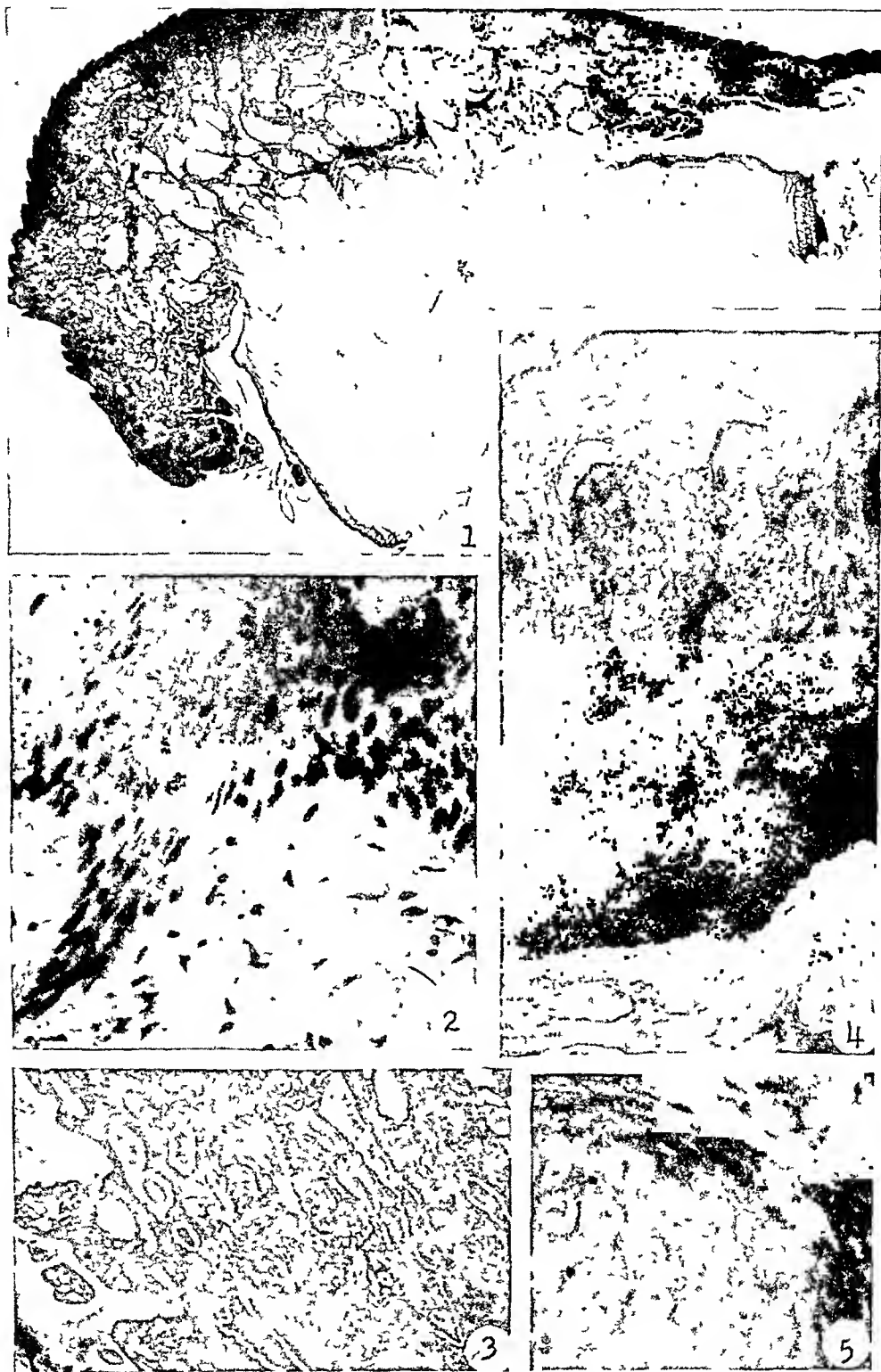
All the tissues were fixed for four to six hours in formaldehyde-Zenker solution,⁴ embedded in pyroxylin (nitrocellulose) and cut serially at 8 to 12 microns. At first all the tissues were stained with hematoxylin-eosin-azure II, Mallory-azocarmine,⁵ orcein and phosphotungstic acid-hematoxylin. The phosphotungstic acid-hematoxylin staining was discontinued since it did not demonstrate elastic fibers as well as did staining with orcein, collagen fibers as well as did Mallory azocarmine staining or reticular fibers as well as did silver impregnation. Many of the tissues were also impregnated with silver by a modified Bielschowsky technic, and some of the latter were counterstained by the Mallory-azocarmine technic.

GROSS PATHOLOGIC FEATURES OF THE AMPUTATED SPECIMENS

Since little can be added to the careful descriptions recorded by Larrey,^{3a} Friedman^{3c} and Lesser,^{3c} there is no necessity to enter into any detailed analysis of the results of the pathologic examination of the gross specimens. Regardless of how much tissue was lost, each of the amputation specimens was made up of a mummified gangrenous zone and a reactive or regenerative zone, usually separated by a demarcation zone.

4. This is Zenker's solution to which, instead of acetic acid, solution of formaldehyde U.S.P. has been added to a concentration of 10 per cent.

5. This is Mallory's connective tissue stain with azocarmine G or B substituted for acid fuchsin.



(See legend on opposite page)

In the first zone the skin was horny and black and extended a variable distance into the toe or the foot; beneath it was found an odorless, slightly moist tissue. When the bone protruded, it was hard and dry. If the bone was buried in gangrenous tissue, it usually had a purple discoloration and a wet appearance, most marked at the epiphyses. Occasionally, when the black, gangrenous tissue was only a few millimeters thick, the tissue in the center of the toe was grossly normal.

The demarcation zone was rather softer and moister than the other two zones and made an indentation in the surface of the skin. It was odorless in the great majority of cases, since the amputations were performed before frank secondary infection had begun.

The third zone, which was only a few centimeters in length, was usually slightly erythematous and just slightly edematous. Thereafter, erythema and edema disappeared. The unamputated portions greatly resembled the feet of a patient who had not lost tissue, and, in general, those who had had amputations suffered from the same clinical symptoms as did those who had lost no tissue. Surprisingly, the severity of these symptoms was greatest in the group without loss of tissue.⁶

HISTOLOGIC DESCRIPTION

GANGRENOUS ZONE

Although the general histologic features, especially the fiber structure, were preserved, the tissues of the gangrenous zone had become desiccated and had lost their normal ability to be stained differently (figs. 1 and 3). The dead nuclei, but not the cytoplasm, were clearly seen (fig. 2). In the less severely damaged areas, the dead cells and fibers were well preserved, and, except for some hyalinization of the tissue, there was little distortion (figs. 3 and 4).

Epidermis.—In the more severely mummified areas the epidermis was a hyalinized structureless mass without even the normal amount of intercellular and intracellular fluid. When nuclei were still present, they were found as pyknotic masses in the stratum germinativum, especially near the sweat ducts (fig. 2). In the less severely mummified areas there were intercellular and extracellular vacuoles (fig. 4). No leukocytic infiltration or signs of regeneration were seen. The basement membrane was well preserved (fig. 3).

Sweat Glands.—The nuclei of the cells forming the sweat glands were pyknotic but still stainable. In silver impregnations the hyalinized cytoplasm and the surrounding hyalinized connective tissue were seen to be separated from each

6. Block.¹ Kräuse.^{2a}

Fig. 1.—Low power view of an amputated toe illustrating the three zones: gangrene, demarcation and reaction. The viable epidermis is undercutting the gangrenous tissue. (Hematoxylin-eosin-azure II; $\times 6$; Army Institute of Pathology negative 98470.)

Fig. 2.—Gangrenous area with dead, pyknotic nuclei clearly visible. (Hematoxylin-eosin-azure II; $\times 400$; Army Institute of Pathology negative 98483.)

Fig. 3.—Essentially normal reticular fiber basement membrane and normal reticular fiber scaffolding. The fibers are collapsed on one another because of the desiccation. (Silver; $\times 115$; Army Institute of Pathology negative 98474.)

Fig. 4.—Epidermal intracellular vacuolation and intercellular fluid in gangrenous but not severely mummified tissue. The vessels are dilated and obstructed by red cells. (Hematoxylin-eosin-azure II; $\times 185$; Army Institute of Pathology negative 98481.)

Fig. 5.—Sclerotic but not mummified nerve. Collagen stains with aniline blue. (Mallory-azocarmine; $\times 450$; Army Institute of Pathology negative 98485.)

other by a basement membrane (fig. 3, upper right). In a few cases the sweat glands underwent granular degeneration, most marked in the secretory portions. This degenerative process began as an accumulation of azocarmophilic granules similar to secretory granules in the apical ends of the cells. Then the nuclei degenerated, and the granules filled the cells until finally the gland was transformed into a mass of granules, still separated by an argyrophil basement membrane from the adjoining hyalinized connective tissue (fig. 3).

Nerves.—Many of the nerves were sclerosed (fig. 5); in some cases this was preceded, in others followed, by mummification. The sclerosis consisted of an increase in the endoneurial collagen. The onset of mummification was evidenced by a change in the staining by the Mallory-azocarmine technic. In partially mummified areas the collagen was stained, part by the aniline blue and part by the azocarmine (fig. 6*b*). In most severely mummified areas the whole nerve stained diffusely with azocarmine, but by means of silver impregnation one could demonstrate a fairly normal reticular fiber network, indicating that the reticular fiber scaffolding was still largely intact.

Dermis.—*Papillary Layer:* The papillary folds were usually wide, but this depended on the extent of dilatation of the vessels. With the Mallory-azocarmine technic, this layer showed a diffuse red stain, indicating mummification. Characteristically, there was rarely evidence of inflammation (figs. 1 to 4). The nuclei were pyknotic and elongated (fig. 2) and had undergone degeneration or decreased in number.

The most spectacular manifestation of injury was a massive dilatation of most of the blood vessels, so that it was impossible to differentiate arterioles from venules, because of compression of the walls. The lumens were filled with packed masses of erythrocytes, many of which were hemolyzed. There was no attempt at reorganization of these occluded vessels.

The reticular fibers were preserved, and perhaps slightly thickened (fig. 3). The collagenous fibers were hyalinized, usually mummified, and compressed on one another (fig. 2). The elastic fibers were rather variable in thickness and distribution.

Reticular Layer: In general, the changes seen here were the same as those described in the papillary layer. Owing to the presence of numerous venous sinusoids, the vascular dilatation was more spectacular here than in the papillary layer (figs. 3 and 4). However, even in these vessels a compressed but fairly normal reticular fiber network was found, although the collagenous fibers failed to stain normally with the Mallory-azocarmine technic. The mummification never proceeded as a wave advancing from superficial to deep tissues, but always began as patchy, focal areas. The elastic fibers were variable—some thick, others thin.

Glomuses were not seen. In spite of a careful review of the literature, only one reference to the appearance of the glomus in cold injury was noted.⁷

Subcutaneous Tissues.—*Fat:* The normal structure of the subcutaneous tissue was preserved (fig. 1). There was sometimes thickening of the collagenous fibers and reticular fibers around the fat lobules and fat cells. This intercellular tissue was often mummified. The process of sclerosis and mummification was most severe between the fat lobules and at the periphery of the lobule (fig. 8). Except near the zone of demarcation, there was no inflammation. Had it not been for occasional absence of nuclei, the dead fat cells would have looked almost normal.

Subcutaneous Vessels: The neurovascular septums were thickened and mummified. The vessels were greatly distended and filled with masses of red blood

7. Theis, F.: Arch. Phys. Therapy 21:663, 1940.

cells. The mummification involved the smooth muscle cells first and to the greatest degree. In the most advanced cases the walls of the vessels were fused into a homogeneous mass with a few degenerated nuclei. In these cases the orcein-stained slides or the silver-impregnated slides often were useful in differentiating arteries from veins, since the elastic and reticular fibers were usually less severely involved than the other tissue components. (fig. 7).

Striated Muscle.—The mummification involved the muscle fibers more than it did the intermuscular connective tissue, which was increased in amount. The reticular fibers were well preserved. The most noticeable changes were a progressive but patchy hyalinization and a decrease in number of nuclei of the muscle cells.

Bone.—The bone spicules had undergone aseptic necrosis. In covered bones the marrow was a mass of granular debris which still suggested the basic structure of marrow, but in bones that protruded through the flesh the marrow had disappeared completely.

DEMARICATION ZONE

This zone, which consisted of a mass of degenerated leukocytes, divided the other two zones (fig. 1). Here the fiber scaffolding of the tissue had been destroyed. If this zone was diffuse and without intense leukocytic infiltration, the destruction of the fibers was incomplete (fig. 8). The demarcation zone occurred at any angle to the surface skin. It was no respecter of tissue layers and even cut through individual vessels (fig. 8). It was more closely adherent to the gangrenous zone than to the reactive zone, since it tended to split from the latter in sectioning.

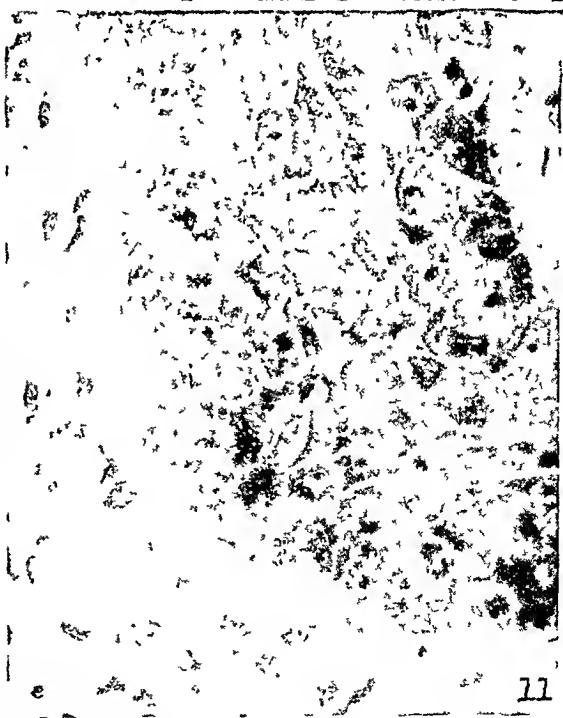
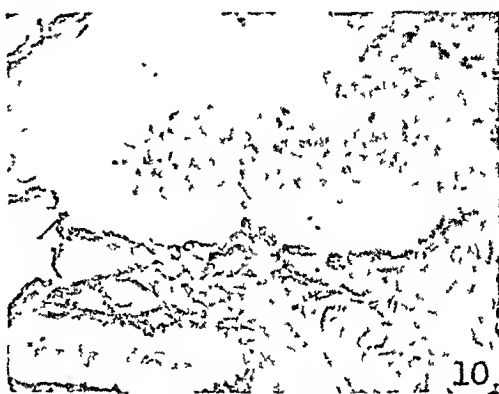
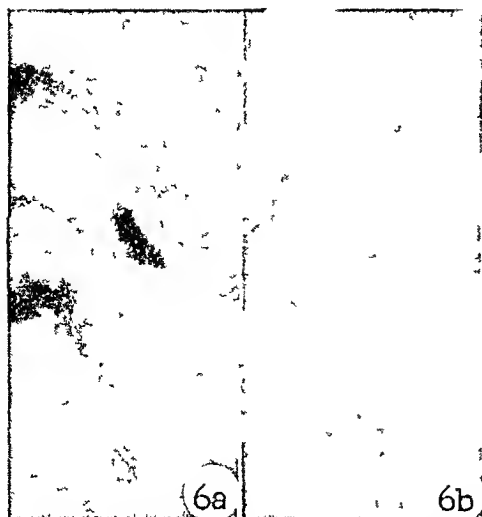
REACTIVE OR REGENERATIVE ZONE

There was a tremendous variation in the extent of pathologic change in this zone from patient to patient and even in the same patient. Entirely normal structures were often found. In no single patient was the whole regenerative process completely represented.

Epidermis.—Three fairly clear areas were seen as the epidermis was traced from the amputation line distally to the zone of demarcation (figs. 1, 9, 10, 11, and 16).

Distally the epidermis in this zone consisted of a few layers of rather flat epidermal cells, with clear intercellular bridges, growing out like flat tongues. There was little differentiation of the stratum granulosum or the stratum lucidum, but a covering of flat squamæ was present (fig. 9). This flat epidermal tongue undercut the demarcation zone, tending to separate the gangrenous and demarcation zones from the viable tissue (fig. 1). The basement membrane was clearly demonstrable up to the point where the epidermal "tongue" ended as a few irregular stellate cells (fig. 10). The underlying connective tissue was fibrous and resembled the reticular layer more than the papillary layer (fig. 10). In rare instances the epidermis had grown past the viable tissue and was found resting on a substrate of dead papillary tissue (fig. 10).

Just proximal to the flat epidermal "tongue" was an area with hyperplastic epidermal rete pegs (figs. 1 and 11). The cells were swollen and had numerous mitoses and vesicular nuclei. The mitoses were located in the rete pegs (fig. 11), not in the surface epithelium. Binucleate cells were seen. Some of the cells had paranuclear vacuoles. There was a great deal of variation in the degree of hyperplasia of this area from patient to patient, but in general it varied inversely with



(See legend on opposite page)

the amount of collagen in the papillary substrate and directly with the vascularity and edema. The epidermis was always separated from papillary connective tissue by a well defined basement membrane (fig. 24).

The third area, except for mild hyperplastic changes, appeared normal (fig. 16).

Sweat Glands.—There was no clear difference between the secretory and excretory sweat gland cells of the patients and those of the controls except for the degeneration to be described, nor was there unequivocal evidence of any unusual regenerative activity, although in many instances the sweat glands were in areas in which the surrounding structures were in the midst of active regeneration. The total number of sweat glands was within the normal range.

Degenerating ducts were found in about one sixth of the sweat glands. The degeneration was usually found in the superficial third of the reticular layer and did not occur in areas remarkable for either degeneration or regeneration. The degeneration was initiated by cytoplasmic swelling; this was followed by fusion of the cytoplasm of the cells, resulting in a structure resembling a foreign body giant cell (fig. 12). That such structures were not foreign body giant cells was indicated by the observation that at this state inflammatory cells were never found around the sweat glands with these large cells. There was no degenerating material to attract giant cells, and later, when degeneration had set in, only neutrophils were attracted (fig. 13). Fibers were related to the pseudo giant cell in such a way as to suggest a basement membrane separating epithelium from connective tissue.

The next stage of the degeneration was marked by the appearance of large, irregular granules staining similarly to the keratohyaline granules of the epidermis. Finally, pyknosis of nuclei and hyaline clouding of cytoplasm ensued, coinciding with an infiltration of neutrophilic leukocytes.

The somewhat myxomatous periglandular arcolar tissue about the secretory acini not only was devoid of inflammation but was distinctly vascular. The edema was external to the reticular fiber basement membrane but had penetrated the collagenous fiber capsule of the sweat gland acini. In certain patients the elastic fibers of the gland capsules were decreased in number. An abnormal pattern

Fig. 6.—Serial sections of a sclerotic, partially mummified nerve with good preservation of histologic structure. In *b* the collagen stained red and blue, indicating partial mummification. ([*a*] Hematoxylin-eosin-azure II; $\times 1,100$; Army Institute of Pathology negative 98489. [*b*] Mallory-azocarmine; $\times 1,100$; Army Institute of Pathology negative 98487.)

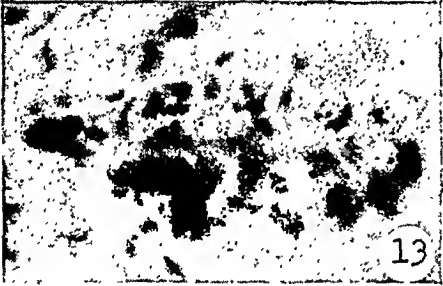
Fig. 7.—Distended subcutaneous artery in the gangrenous zone, with flattened, irregular, discontinuous internal elastic membrane. (Orcein; $\times 70$; Army Institute of Pathology negative 98488.)

Fig. 8.—Junction of the gangrenous and reactive zones passing through an artery. Note the sclerosis around the fat lobules at the lower right. (Mallory-azocarmine; $\times 26$; Army Institute of Pathology negative 98514.)

Fig. 9.—High power view of the distal tip of a flat epidermal "tongue" extending from the right to grow over the avascular papillary substrate. (Hematoxylin-eosin-azure II; $\times 210$; Army Institute of Pathology negative 98495.)

Fig. 10.—Serial section next to that shown in figure 9. The reticular fiber basement membrane is present under the advancing epidermal "tongue" up to the very end of the epidermis. (Silver; $\times 210$; Army Institute of Pathology negative 98493.)

Fig. 11.—Rete pegs in a hyperplastic area of the epidermis, with swollen, hypertrophic cells and numerous mitoses resting on a vascular, mildly inflamed, and edematous papillary layer. (Hematoxylin-eosin-azure II; $\times 500$; Army Institute of Pathology negative 98472.)



(See legend on opposite page)

of elastic fibers was not invariably present in the glands with the most surrounding edema and, in addition, was seen in glands with little surrounding edema.

Nerves.—Generally there was little evidence of pathologic change in the nerves, and often it was limited to edema. It was not unusual to see a normal nerve passing through an area of inflammation. Occasionally, fatty macrophages were seen between nerve fibers or within a nerve. The Schwann cells were a little decreased in number. Most spectacular, when present, was extreme sclerosis, which was so intense that the nerves resembled the glomeruli of chronic glomerulonephritis. The fibrosis began as a thickening of the fine collagenous and reticular endoneurial fibers normally present and progressed to become a mass of wavy connective tissue fibers with an occasional Schwann cell. This then contracted to give the final picture (fig. 14). Vascularization of these areas of whorled neural fibrosis was rarely seen (fig. 15). The neural fibrosis seemed to appear most often in the larger, deeper nerves, but this impression may have been due to the difficulty of recognizing the smaller nerves. No evidence of regeneration (mitosis, increase in number of Schwann cells, cellular hypertrophy) was seen.

Arteries.—The great majority of the arteries were normal. In general, the less change in the surrounding tissue, the more normal were the vessels. The most common pathologic feature was edema, which was part of the generalized edema of the neurovascular septums (fig. 16). In the larger arteries it was most noticeable in the adventitia. A distortion of the normal regular vascular pattern was often seen, especially in the subpapillary areas (fig. 16). The collagen and the reticulum were occasionally thickened, primarily at the inner edge of the media. The internal elastic membrane was sometimes discontinuous in the smaller arteries (fig. 17*a* and *b*); less often new elastic fibers had been deposited on its inner surface, and very occasionally it was shredded. An increase of elastic fibers could be observed in the media in a few instances. In general, proliferative changes of elastic fibers were rare. The arteries were seldom invaded by inflammatory cells and, unlike those in thromboangiitis obliterans, were not matted together with the vein.

By studying serial sections, it was usually possible to demonstrate a sludge of agglutinated red cells distending the lumens of arteries in which the processes

Fig. 12.—Fine collagenous fibers separate the pseudo giant cell from the surrounding connective tissue. (Mallory-azocarmine; $\times 660$; Army Institute of Pathology negative 98502.)

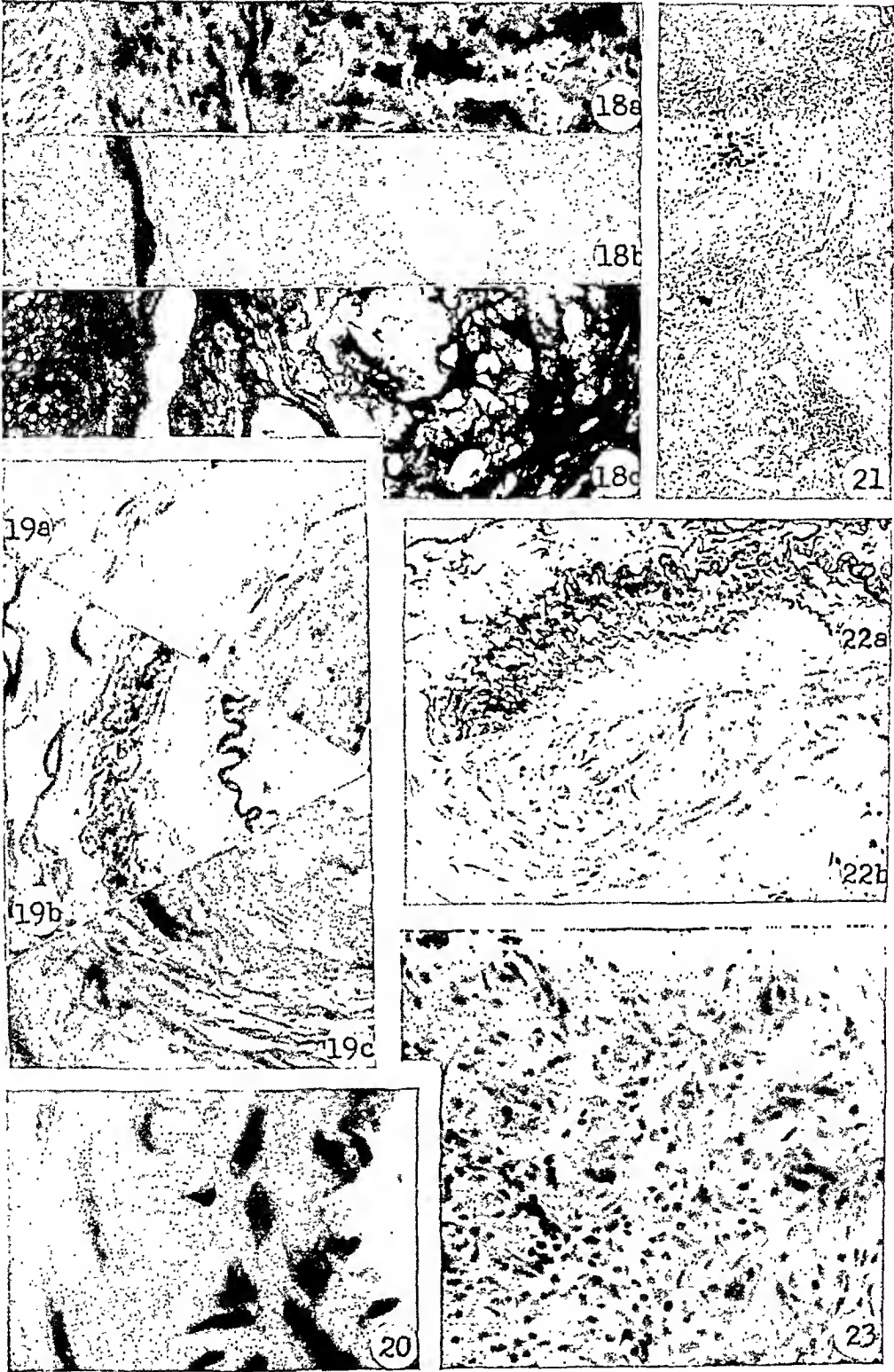
Fig. 13.—Final stage of the destruction of sweat gland ducts with an infiltration of polymorphonuclear leukocytes. (Hematoxylin-eosin-azure II; $\times 550$; Army Institute of Pathology negative 98499.)

Fig. 14.—Stages of the fibrosis of nerves brought about by thickening of endoneurial collagen. The most scarred nerve is at the middle left; the least scarred, at the middle right. (Mallory-azocarmine; $\times 70$; Army Institute of Pathology negative 98835.)

Fig. 15.—Vascularization of an extremely sclerotic nerve. (Hematoxylin-eosin-azure II; $\times 55$; Army Institute of Pathology negative 98501.)

Fig. 16.—Mildly regenerative area with fairly normal epidermis and rete pegs. Note the expansion of neurovascular septums due to edema, mild inflammation and increased number of vessels. (Hematoxylin-eosin-azure II; $\times 48$; Army Institute of Pathology negative 98833.)

Fig. 17.—Recanalized small artery in a mildly inflamed neurovascular septum. Note the original internal elastic membrane, irregular, discontinuous and flattened, between the new and the original media. [*a*] Orcein; $\times 600$; Army Institute of Pathology negative 98630. [*b*] Hematoxylin-eosin-azure II; $\times 600$; Army Institute of Pathology negative 98669.)



(See legend on opposite page)

of organization and canalization had not progressed. Occasionally the sludge was found to be invaded by granulation tissue (fig. 18). From the very beginning, reticular and collagenous but never elastic fibers were found in this rather vascular granulation tissue. Granulation tissue was but rarely seen invading the red cell sludge in a vessel whose walls were dead. The vascularity of this invading tissue was much greater than that found ordinarily in primary intimal proliferation. The internal elastic membranes were usually flattened (fig. 18*b*), indicating that the process had been initiated in vessels that were once paralytically dilated. It was often difficult to recognize the smaller arteries at this stage, because the media was irregular, but a study of the fiber preparation, especially the elastic fibers, in the serial sections was usually helpful.

The further development of the canalization consisted of a swelling of the fibroblasts of the granulation tissue and differentiation of some of them into smooth muscle cells (figs. 19 and 20). In one section this occurred about one of the capillary loops while the original lumen was still filled with agglutinated red cells. Characteristically, these new muscularis cells were swollen and epithelioid in appearance and had poorly developed reticular and collagenous support (fig. 20). An internal elastic membrane had not formed inside this new muscularis (fig. 19*b*). Fibrosis had often occurred in the original muscularis (fig. 19*c*). The internal elastic membrane was usually not thickened or split. A patch of myxomatous change was sometimes seen in the remains of the original intima, and all the other changes previously described (edema, dilatation) were also found. The media was replaced by granulation tissue with iron-containing macrophages in some of the arteries adjacent to the demarcation zone.

In 2 instances a special type of degeneration was seen, consisting of the replacing of the smooth muscle cells by a mass of azocarminophilic granules. This occurred only adjacent to the demarcation zone where an otherwise normal artery would be found ending abruptly at the zone. The fiber pattern of these vessels was normal.

Veins.—A surprisingly high percentage of the veins were also normal, especially of those distant from the demarcation zone, where the other pathologic changes

Fig. 18.—Serial sections of a large artery illustrating an early stage of the invasion of the red cell sludge. Elastic fibers are present only in the flattened internal elastic membrane of the original dilated artery. ([*a*] Hematoxylin-eosin-azure II; \times 255; Army Institute of Pathology negative 98508. [*b*] Orcein; \times 255; Army Institute of Pathology negative 98509. [*c*] Silver; \times 255; Army Institute of Pathology negative 98510.)

Fig. 19.—Serial sections of the same recanalized artery with new muscularis inside the original internal elastic membrane. ([*a*] Hematoxylin-eosin-azure II; Army Institute of Pathology negative 98519. [*b*] Orcein; \times 315; Army Institute of Pathology negative 98556. [*c*] Mallory-azocarmine; \times 315; Army Institute of Pathology negative 98517.)

Fig. 20.—Higher power magnification of the section shown in figure 19 *a*, to illustrate the epithelioid swollen muscle cells of the new media. (Hematoxylin-eosin-azure II; \times 900; Army Institute of Pathology negative 98555.)

Fig. 21.—Edematous neurovascular septums with dilated veins and mononuclear inflammatory cells. (Hematoxylin-eosin-azure II; \times 62; Army Institute of Pathology negative 98629.)

Fig. 22.—Serial section of a recanalized vein with elastic fibers present only in the original intima-media. ([*a*] Orcein; \times 235; Army Institute of Pathology negative 98712. [*b*] Hematoxylin-eosin-azure II; \times 235; Army Institute of Pathology negative 98713.)

Fig. 23.—Angiomatous granulation tissue. (Hematoxylin-eosin-azure II; \times 225; Army Institute of Pathology negative 98506.)

were likewise minimal or absent. Edema, irregularly of vascular pattern, increase in number and generalized mild dilatation were the changes most often encountered in veins, especially in the more superficial layers (fig. 16). The perivenous mononuclear infiltration varied with the extent of inflammation, and when it was extensive, mononuclears invaded the walls of the vein (fig. 21). In the larger veins fibrosis of both intima-media was often found.

A spectacular recanalization, the labyrinthine endophlebitis of Friedman,^{3e} was demonstrable. In the veins one could rarely find, as one could in the arteries, the earliest stage of the process—that is, the invading of the red cell sludge by granulation tissue. Usually what was seen was the distended vein with much of its original lumen filled with granulation tissue. In such instances the orcein preparations were invaluable in demonstrating the site of the original intima-media.

In the most advanced stages (fig. 22) a few scattered smooth muscle cells could be seen about the lumen of the vein, external to that a space filled with myxomatous connective tissue, and finally a network of smooth muscle. Collagenous and reticular fibers were interwoven in the two muscle layers, but elastic fibers were well developed only about the outer original muscle layer. Occasionally, smooth muscle could be distinguished around several capillaries in the proliferated intima of a single vein, and the smooth muscle of the original intima-media was disrupted, so that the true original structure was recognized only by tracing the vessel through the series of different stains. In practically every case in which a few small capillaries and veins were found in close proximity in a myxomatous tissue, a vein was being recanalized.

Connective Tissue Proper.—The connective tissue proper had undergone varying degrees of pathologic change. In general, edema, numerous inflammatory cells and dilated vessels were found under hyperplastic epidermis and near the demarcation zone (figs. 15, 16 and 17); near the amputation line the connective tissue was less active.

In many cases the granulation tissue was so vascular that it resembled an angioma, particularly in areas where there was no evidence of recanalization of preexisting vessels and in close proximity to hyperplastic epidermis (fig. 23). The granulation tissue was vaguely reminiscent of Kaposi's sarcoma. The angiomatous areas were composed of numerous small irregular capillaries with closely adherent perivascular mesenchymal cells. Between the capillaries was a diffuse scattering of mononuclears and fibroblasts. All of the cells were hypertrophic. The perivascular mesenchymal cells bore essentially the same relationship to the capillaries as the embryonic mesenchyme does to the endothelium in the differentiation of arteries and veins. Elastic fibers were never found closely related to the newly formed capillaries in these angiomatous areas, but each capillary was surrounded by a network of reticular and collagenous fibers.

Papillary Layer: Mild edema was found, as well as slight but universal dilatation of the capillary loops (figs. 11, 16 and 24). The reticular fibers were teased apart by the mild edema (fig. 24), and the elastic fibers were irregular and decreased in number. The basement membrane was always well preserved (fig. 24). Under the more hyperplastic epidermis, the edema, mononuclear infiltration, cellular hypertrophy and capillary dilatation were more marked. In some instances, numerous mast cells were seen developing from fixed macrophages. In general, neutrophils were rare or absent. In 1 case numerous eosinophils were observed. Under the advancing epidermal "tongue" previously described, the papillary tissue was fibrous and avascular (figs 9 and 10).

Reticular Layer.—The most common finding was an expansion of the neurovascular septums, which was caused by edema, an increased number of vessels, fibers and inflammatory cells, and, occasionally, foci of subacute inflammation (figs. 16 and 21). Under low power magnification the reticular layer seemed to be divided into little areas of fibrous tissue, separated from one another by the hyperplastic, edematous neurovascular septums. The collagenous fibers between the septums were slightly sclerotic and sometimes appeared as small sclerotic whorls between the neurovascular septums. The elastic fibers were irregular, and in the more actively inflamed areas they were entirely absent. Even in the tissue sections in which pathologic changes were minimal, edema and vasodilatation were present.

Subcutaneous Layer.—The changes in this layer were identical with those in the reticular layer except for the presence of the fatty tissue (fig. 25). The interlobular fat septums were expanded in the same manner as the neurovascular septums of the reticular layer. Intercellular edema was marked and mononuclear infiltration minimal. Epithelioid masses of fatty macrophages, resembling fetal fat, often separated the fat cells, but it was possible, as in the bone marrow, to demonstrate transitional forms from inflammatory mononuclears (polyblasts of Maximow) to numerous vacuolated fatty macrophages. The polyblast cytoplasm hypertrophied and became vacuolated while the nucleus became lighter and the chromatin more delicate (figs. 29 and 30). Also, the reticular and collagenous fibers were arranged around the fat cells in such a way as to suggest that the fatty macrophages had penetrated between the fat cells and were not derived from the latter, since each fatty macrophage did not have its own reticular fiber sheath as did the fat cells (fig. 25).

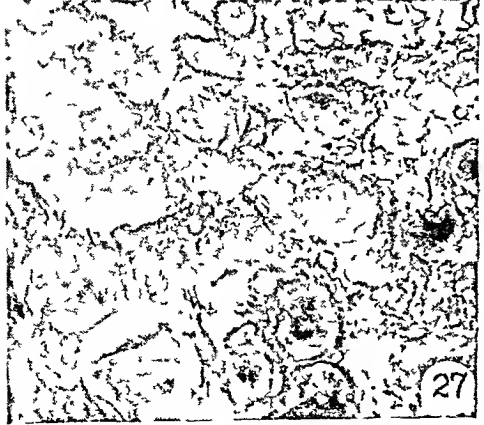
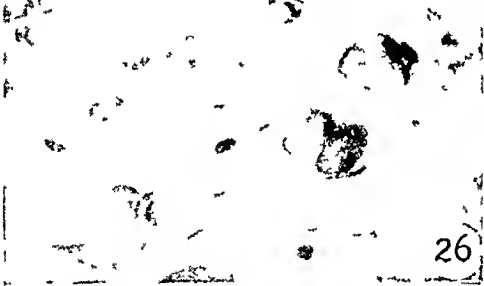
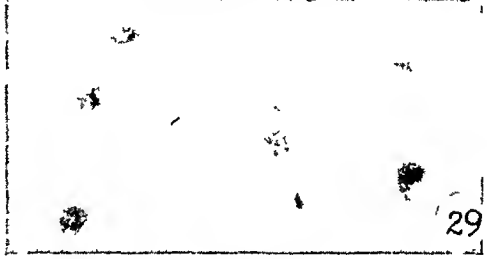
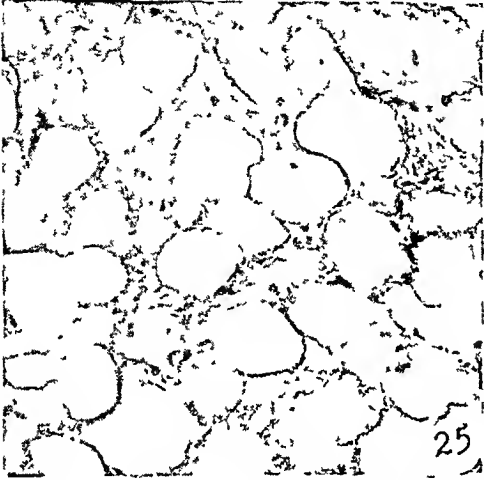
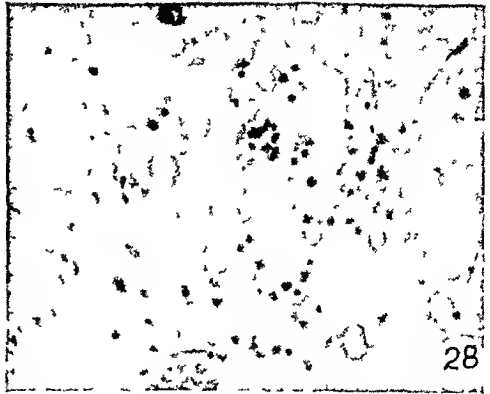
Striated Muscle.—Where there was extreme hyperplasia of muscle nuclei, the normal cytoplasmic striation was indistinct. Many of the nuclei were swollen and lobate. In some areas there were long successions of fibers with excessive numbers of nuclei. There was no evidence that inflammatory cells invaded the muscle fibers (fig. 26). The reticular and collagenous fiber sheaths were normal, but the fibers were separated by an edematous tissue with sparse reticular and collagenous fibers and inflammatory mononuclears (fig. 27).

Bone and Marrow.—In general, there were two types of marrow in the reactive zone, the somewhat fibrous marrow adjoining the gangrenous tissue and the aplastic marrow at some distance from the gangrenous tissue.

In the latter type there was gelatinous degeneration of the marrow similar to that of the aplastic stage which follows irradiation and nitrogen mustard therapy (fig. 28). The marrow was replaced by a basophilic colloid-like material. The smaller vessels were distended with red blood cells. Although no lesion was seen in the vessel walls, some intact red cells were scattered diffusely through this gelatinous marrow.

The predominant cell in the gelatinous aplastic marrow was a fibroblast-like reticular cell, cytologically identical with the reticular cell of the bone marrow. Because of the marked decrease in free hemopoietic cells (fig. 28), these reticular cells were prominent, although there was no evidence of an absolute increase in their number. There were also among the reticular cells occasional fixed macrophages, a few of which had numerous fat vacuoles. The osteoblasts were numerous and lined most of the bone spicules but were probably within numbers normal for the age of the patients. Some of the osteocytes appeared viable.

The free cells were primarily mononuclear inflammatory cells (hypertrophied lymphocytes and monocytes, free macrophages, plasma cells) and fatty macro-



(See legend on opposite page)

phages of the type seen in the subcutaneous fat lobules. These macrophages, as in the subcutaneous tissues, were derived from the mononuclears by hypertrophy and fat storage in the latter (figs. 29 and 30). No hemocytoblasts or erythroblasts and only a few isolated megakaryocytes were seen. There were a few scattered small eosinophilic and neutrophilic myelocytes with dark nuclei, not resembling too closely the normal human myelocytes. The usual transitional forms connecting the myelocytes to hemocytoblasts were missing. Collagenous and reticular fibers were essentially normal.

Gelatinous degeneration, vessels distended with erythrocytes, and hemopoietic aplasia were also found in the more fibrous marrow, but the collagenous and reticular fibers, as well as the free and fixed lipophages, were much more numerous. In a sense this part of the marrow corresponded to a demarcation zone. The osteoblasts here were also numerous but were unusual in that they were surrounded by a dense feltwork of collagenous fibers, continuous both with that of the marrow and with the matrix of the adjoining bone spicules (fig. 31). It was not possible to determine whether these collagenous fibers were calcified, since no specific staining for calcium was done. Many of the osteoblasts were in the process of being surrounded by the new collagen and so becoming osteocytes. Characteristically, this new osteoid tissue was laid down on the old necrotic bone without any evidence of preceding resorption of necrotic bone.

COMMENT

GANGRENOUS ZONE

Although there has been general recognition of the presence of three general types of pathologic change corresponding to the gangrenous, demarcation and reaction zones seen grossly, little attention has been paid to the significance of the functions of each zone. Reference has been

Fig. 24.—Numerous dilated vessels, normal basement membrane, and reticular fibers teased apart by edema in the papillary layer under hyperplastic epidermis. (Silver; \times 295; Army Institute of Pathology negative 98709.)

Fig. 25.—Pale lipophages in the upper center; reticular fibers investing individual fat cells at the lower left. (Mallory-azocarmine; \times 440; Army Institute of Pathology negative 98705.)

Fig. 26.—Multinucleated hyperplastic muscle cells with absence of striations. (Hematoxylin-eosin-azure II; \times 400; Army Institute of Pathology negative 98606.)

Fig. 27.—Normal perimascular fibrous reticulum with increased number of intermuscular reticular fibers and intermuscular edema. (Silver; \times 395; Army Institute of Pathology negative 98825.)

Fig. 28.—Gelatinous aplasia of marrow, dilatation of vessels and scattering of mononuclear cells. (Hematoxylin-eosin-azure II; \times 210; Army Institute of Pathology negative 98475.)

Fig. 29.—Stages of the process by which lymphocytes are transformed into fatty macrophages. The cell showing the least hypertrophy is at the lower left. (Hematoxylin-azure II; \times 660; Army Institute of Pathology negative 98828.)

Fig. 30.—End stage of the formation of fatty macrophages. These cells are identical with those seen in the fatty subcutaneous tissue. (Hematoxylin-eosin-azure II; \times 1,100; Army Institute of Pathology negative 98827.)

Fig. 31.—New bone forming around osteoblasts. The necrotic bone at the top was stained red by the azocarmine, contrasting with the blue-stained viable collagenous fibers. (Mallory-azocarmine; \times 400; Army Institute of Pathology negative 98476.)

made to the variation in pathologic change from zone to zone,⁸ but only Wieting,³¹ Staemmler⁹ and Siegmund^{10a} have emphasized that most of what is seen in the reaction zone is not the true change resulting from exposure to moist cold, and they advised that the early acute stage of trench foot be studied to determine the true pathologic features of trench foot. Siegmund¹⁰ expressed the belief that the reaction zone, to which by far the greatest attention had been paid, represented a non-specific reaction to injury. Probably, however, it represents a mixture of the primary degenerative and secondary reparative processes. The gangrenous zone, on the other hand, represents the true structure of the tissues at the time they undergo mummification, preserved by desiccation.

Only rarely has tissue been available from trench foot or immersion foot in the early stages.¹¹ Although there are minor differences, the descriptions are essentially similar. In particular, the pathologic changes observed in the acute stages in Friedman's series^{3e} closely resemble those found in the gangrenous zone in the present study and those observed in the same zone by Kriege¹² over fifty years ago. The diffuse degeneration and mummification with loss of differential staining qualities of the gangrenous zone combined with preservation of the gross histologic structure have been noted.¹³ Adami and Nichols¹⁴ and Ziegler¹⁵ have separated the primary gangrenous degeneration from the secondary desiccation of the gangrenous tissues. The cells are much more susceptible than the fibers.

Although the normal fiber pattern was usually fairly well preserved, the staining qualities of the collagenous fibers, and in consequence presumably their chemical and/or physical condition, had changed. Gen-

8. (a) Hodara, M.: *Monatsh. f. prakt. Dermat.* **22**:445, 1896. (b) Böttcher, H.: *Virchows, Arch. f. path. Anat.* **312**:464, 1944; (c) Hellmuth, M.: *Arch. f. klin. Chir.* **158**:702, 1930. (d) Patterson and Anderson.^{3d}

9. Staemmler, M.: (a) *Virchows Arch. f. path. Anat.* **312**:501, 1944; (b) *Zentralbl. f. Chir.* **69**:1757, 1942.

10. Siegmund, H.: (a) *Zentralbl. f. Chir.* **70**:1558, 1943; (b) *Jahresk. f. ärztl. Fortbild.* **34**:439, 1943.

11. (a) Ducuing, J.; D'Harcourt, J.; Folch, A., and Bonfill, J.: *J. de chir.* **55**:385, 1940. (b) Blackwood, W.: *Brit. J. Surg.* **31**:329, 1944. (c) Friedman.^{3e,f} (d) Wieting.³¹ (e) Staemmler.⁹

12. Kriege, H.: *Virchows Arch. f. path. Anat.* **116**:64, 1889.

13. (a) Hecht, V.: *Wien. med. Wchnschr.* **65**:1487, 1915. (b) Lange, K., and Boyd, L.: *Surg., Gynec. & Obst.* **80**:346, 1945. (c) Greene, R. J.: *J. Path. & Bact.* **55**:259, 1943. (d) Friedman.^{3e} (e) Hellmuth.^{8c} (f) Ducuing and others^{11a} (g) Kriege.¹²

14. Adami, J. G., and Nichols, A. G.: *Principles of Pathology*, Philadelphia, Lea & Febiger, 1908-1909, vol. 1.

15. Ziegler, E.: *General Pathology*, translated by A. H. Buck, New York, William Wood and Company, 1895.

eralized fibrosis of tissue has been noted,¹⁶ but whether reference was made to the gangrenous zone specifically has not been clear. The slight changes occurring in elastic fibers have been described by Hodara.^{8a} Attention has not been directed to the remarkable resistance of the reticular fibers and in particular to the resistance of the reticular fiber basement membrane.

— That degenerative changes do not become complete until about ten days after the vasoconstrictive phase has been borne out in these cases by the interval which elapsed before the appearance of gross gangrene.¹⁷ The delay between exposure and death of the tissues was sufficient for deposition of collagen, the only proliferative change seen, to have occurred in the nerves (fig. 14). Siegmund^{10a} proposed that the slowing of metabolic processes was the basic reason for the slow onset of degeneration after the vascular damage in trench foot. This thesis is supported by the observation that cooling of anemic tissues will delay the onset of degeneration¹⁸ or inflammation.¹⁹

By an experimental approach²⁰ it has been shown that cooling a limb readily incites the hemolysis that was so evident in this study and in others.²¹

Numerous investigators have studied the early reactions of experimental animals subjected to cold as a substitute for the human reactions of the acute primary stage of trench foot. However, many of them²² have used acute application of extreme cold rather than chronic exposure to temperatures about the freezing point in a moist environment.²³ It is probable that the results of the latter group of investigators

16. (a) Marchand, F.: *Allgemeine Etiologie*, in Krehl, L., and Marchand, F.: *Handbuch der allgemeine Pathologie*, Leipzig, S. Hirzel, 1908 (b) Friedman,^{3e,f} (c) Siegmund.¹⁰ (d) Greene.^{13c}

17. (a) Nagelsbach, E.: *München. med. Wehrsch.* **66**:353, 1919. (b) Page, M.: *Brit. M. J.* **2**:386, 1914. (c) Berson, R., and Angelucci, R.: *Bull. U. S. Army M. Dept.*, 1944, no. 77, p. 91. (d) Sonnenburg, E., and Tschmarke, P., in von Bruns, P.: *Neue deutsche Chirurgie*, Stuttgart, F. Enke, 1915, vol. 17, p. 1. (e) Block.¹ (f) Lesser.^{3e} (g) Ducuing and others.^{11a}

18. Brooks, B., and Duncan, G. W.: *Ann. Surg.* **112**:130, 1940.

19. Bruneau, J., and Heinbecker, P.: *Ann. Surg.* **120**:716, 1944.

20. Reineboth and Kohlhardt: *Deutsches Arch. f. klin. Med.* **65**:192, 1899.

21. Kriege. ¹² Hecht.^{13a} Greene.^{13c}

22. (a) Uschinsky, N.: *Beitr. z. path. Anat. u. z. allg. Path.* **12**:115, 1892. (b) Rotnes, P., and Kreyberg, L.: *Acta path. et microbiol. Scandinav.*, 1932, supp. 11, p. 162. (c) von Manteuffel, Z.: *Zentralbl. f. Chir.* **29**:65, 1902. (d) Rischpler, A.: *Beitr. z. path. Anat. u. z. allg. Path.* **28**:541, 1900. (e) Kriege.¹² (f) Lange and Boyd.^{13b} (g) Greene.^{13c}

23. (a) Large, A., and Heinbecker, P.: *Ann. Surg.* **120**:707, 1944. (b) Denny-Brown, D.; Adams, R. D.; Brenner, C., and Doherty, M.: *J. Neuropath. & Exper. Neurol.* **4**:305, 1945. (c) Smith, J.; Ritchie, J., and Dawson, J.: *J. Path.*

are pertinent to the problem of the genesis of trench foot, although Greene²⁴ expressed the belief that trench foot is merely a slow development of frostbite.

One must remember that the rat tail (used extensively experimentally) may not be comparable with the human foot, since it is usually impossible to produce severe gangrene in the tail before the animal itself dies of exposure, even though an attempt is made to protect it and just expose the tail.²⁵ In human trench foot and immersion foot, gangrene occurs long before the organism as a whole succumbs to the systemic effects of cold.

Epidermis and Sweat Glands.—The development of the degenerative hyaline changes of the epidermis and the appendages of the skin has been described.²⁶ Clinically it was one of the most obvious signs of gangrene. Uschinsky^{22a} was the only investigator who thought that the epidermis in immediate proximity to the sweat glands was more susceptible than the rest of this tissue. In the present study the sweat glands appeared to show less intense change than did the epidermis. In experiments on the rat tail Blackwood and Russell,^{23d,e} unlike Böttcher,^{8b} were unable to demonstrate gangrenous changes in the epidermis.

Siegmund¹⁰ expressed the belief that the intracellular epidermal vacuoles, which most of the authors have described, are artefacts due to thawing, but it is generally accepted that they are manifestations of the early edema or of damage of a milder type.²⁷ Clinically the vesiculation or the edema is always present at some time.²⁸ It seems probable that the lack of vesiculation observed in the more mummified epidermis is due to the desiccation which follows the gangrene, producing the pic-

& Bact. 20:159, 1915. (d) Blackwood, W., and Russell, H.: Edinburgh M. J. 50:385, 1943; (e) 52:160, 1942. (f) Böttcher.^{8b} (g) Reineboth and Kohlhardt.²⁰

24. Greene, R. J.: Lancet 2:689, 1941.

25. Böttcher.^{8b} Blackwood and Russell.^{23d,e}

26. (a) Davis, L.; Scarff, J.; Rogers, N., and Dickinson, M.: Surg., Gynec. & Obst. 77:561, 1943. (b) Dittrich, O.: Arch. f. Dermat. u. Syph. 157:1, 1929. (c) Fuerst, E.: Beitr. z. path. Anat. u. z. allg. Path. 24:415, 1898. (d) Friedman.^{3e} (e) Theis.⁷ (f) Hodara.^{8a} (g) Böttcher.^{8b} (h) Hellmuth.^{8c} (i) Kriege.¹² (j) Greene.^{13c}

27. Hodara.^{8a} Böttcher.^{8b} Siegmund.¹⁰ Rischpler.^{22d} Uschinsky.^{22a} Smith and others.^{23c} Davis and others.^{26a}

28. (a) Osborne, J., and Cowen, J.: Lancet 2:204, 1945. (b) Edwards, J.; Shapiro, M., and Ruffin, J.: Bull. U. S. Army M. Dept., 1944, no. 83, p. 58. (c) Ungley and Blackwood.^{3b} (d) Lesser.^{3c} (e) Patterson and Anderson.^{3d} (f) Friedman.^{3e} (g) White.^{3h,1} (h) Wieting.³¹ (i) Wright and Allen.^{3m} (j) Staemmler.^{9a} (k) Sonnenburg and Tschmarke.^{17d}

ture of mummification seen in the absence of secondary infection (sphacelization of the older authors²⁹).

It is significant that Rischpler^{22a} and Uschinsky,^{22a} who subjected their animals to severe cold, have stressed the direct effect exercised by this cold on the epidermal cells, while Smith, Ritchie and Dawson^{23c} and Böttcher^{8b} have concluded from experiments which more closely duplicated the process resulting in trench foot that the epidermal degeneration is secondary to the vascular damage.

Nerves.—There is ample evidence of an effect of depression of the environmental temperature on the function³⁰ and the structure³¹ of the nerves. Bickford^{30a} and Denny-Brown and associates^{23b} have demonstrated by functional tests that the various functional modalities are selectively inhibited at different temperatures. Furthermore, Denny-Brown has demonstrated the early involvement of the function and the structure of the myelinated nerves as well as the resistance of the fine vasoconstrictor unmyelinated sympathetic fibers. The conclusion drawn from his work furnish an excellent explanation for the observation that in the stage of reactive dilatation the vessels are still capable of vasoconstriction on stimulation.³²

Surprisingly, the fact that the nerves are involved during the acute stage has not even been mentioned by numerous authors.³³ Presumably they did not see any evidence of early changes in the nerves. Smith, Ritchie and Dawson^{23c} found only mild edema, part of the generalized edema, in rabbits under conditions closely simulating trench foot. But it must be emphasized that this group, with the exception of Smith, Ritchie and Dawson, did not use specific histologic methods to demonstrate the degeneration of myelin, the swelling of the neuromuscular spindle and the occasional axon degeneration detectable with the more delicate technics. Also, one must remember that the immediate degenerative changes are seen most clearly only in the acute condition or in animal

29. Larrey,^{3a} Adami and Nichols,¹⁴ Ziegler.¹⁵

30. (a) Bickford, R.: Clin. Sc. 4:159, 1939. (b) Plaschke, S.: Wien. klin. Wchnschr. 29:5, 1916. (c) Wright and Allen.^{3m} (d) Berson and Angelucci.^{17c} (e) Brooks and Duncan.¹⁸ (f) Large and Heinbecker.^{23a} (g) Denny-Brown and others.^{23b} (h) Edwards and others.^{28b}

31. Patterson and Anderson.^{3d} Friedman.^{3e} Böttcher.^{8b} Staemmler.^{9a} Large and Heinbecker.^{23a} Denny-Brown and others.^{23b} Blackwood and Russell.^{23d,e}

32. (a) Hertzman, A., and Roth, L.: Am. J. Physiol. 136:668 and (b) 680, 1942. (c) Grant, R. T.: Heart 15:257 and (d) 281, 1930.

33. (a) Gruber, S.: Beitr. z. path. Anat. u. z. allg. Path. 84:155, 1930. (b) Grant, R., and Bland, E.: Heart 15:385, 1931. (c) Lewis, T.: ibid. 15:177, 1930; (d) 15:351, 1931; (e) Brit. M. J. 2:795, 1941 (f) Grant.^{32c,d} (g) Lange and Boyd.^{13b} (h) Greene.^{13c} (i) Nagelsbach.^{17a} (j) Davis and others.^{26a} (k) Dittrich.^{26b}

experiments and not in the regenerative zone, where the processes of degeneration and regeneration are present together. Certainly, the study of the gangrenous zone pointed to extreme involvement of the nerves.

Blood Vessels.—The papers in the literature concerning the effect of depression of the environmental temperature on the vessels are so numerous that specific references need not be made. Blackwood^{11b} alone failed to find marked vascular reaction in the 2 cases in which he studied an early stage of immersion foot. Blackwood and Russell,^{23d} using the rat's tail under conditions greatly resembling trench foot, have failed to find any vascular lesion, although Böttcher,^{8b} after a similar experiment, reported contradictory results. Unfortunately, most of the work has been done on vessels in the zone of regeneration or at least on vessels which were already the site of reparative processes. Rarely in regard to human material³⁴ has attention been directed specifically to the vessels in the gangrenous zone, in marked contrast to the amount of effort expended on those in the regenerative zone. However, primarily as a result of animal experiments, emphasis has been placed on the phase of reactive dilation with the marked vasodilation and engorgement of the vessels caused by stasis of red blood cells.³⁵ Usually the veins were so distended with red cells that it was impossible to determine whether or not the red cells tended to agglutinate along the valves as described by Friedman^{3e} and so give rise to the labyrinthine endophlebitis so often seen in the reaction zone.

The smooth muscle cells are the first component of the vessel wall to undergo hyalinization,³⁶ a fact amply borne out by a study of the Mallory-azocarmine slides in this study. As in the connective tissue proper, the collagenous fibers were the first of the various fibers to undergo hyalinization. Von Manteuffel^{22c} described degeneration and new building of elastic fibers in arteries, but from his brief description it is difficult to determine to what extent these processes progressed, and whether he was referring to the gangrenous or to the regenerative zone.

Muscle.—The early injury of striated muscle is manifested mainly by a spotty, irregular change in the cross striations,³⁷ which is then replaced by irregular hyalinization of the striated muscle fiber.³⁸ Necrosis occurs

34. Block.¹ Friedman.^{3e,f} Kriege.¹² Hecht.^{13a} Rischpler.^{22d} Smith and others.^{23c}

35. Friedman.^{3e} Hodara.^{8a} Böttcher.^{8b} Hellmuth.^{8c} Siegmund.¹⁰ Lange and Boyd.^{13b} Greene.^{13c} Uschinsky.^{22a} Rotnes and Kreyberg.^{22b}

36. Kriege.¹² Rischpler.^{22d}

37. Staemmler.^{9a} Siegmund.^{10a} Blackwood.^{11b} Rischpler.^{22d} Lange and Heinbecker.^{23a} Smith and others.^{23c}

38. Blackwood.^{11b} Uschinsky.^{22a} Blackwood and Russell.^{23d,e}

in some cases but is always patchy.³⁹ Atrophy of the individual fibers is a comparatively late change.⁴⁰ Most of these investigators have described early intermuscular edema. In contrast to the marked susceptibility of striated muscle to cold observed in the rat tail,²⁵ only slight susceptibility was noted in striated muscle of the rabbit foot.^{23c} It seems probable that, because of the patchy, irregular nature of the degenerative change, it was not due to the direct effect of an external physical agent. Judging from the preservation of the intermuscular fibers, one concludes that the muscle cytoplasm must have been far less resistant than the connective tissue fibers.

Bone, Bone Marrow and Cartilage.—In spite of the extreme susceptibility of the marrow, there has been little in the literature concerning the degree to which bone and marrow are involved in cold injury of any nature. Friedman,^{3e} in the one bone available to him from a trench foot in the early stage, found no abnormality. Among the very early workers,⁴¹ only Rischpler^{22d} gave a complete account. He described nuclear degeneration, dilated vessels and mononuclear infiltration of the marrow as an early response to cold. Blackwood and Russell^{23d,e} did not even mention the bone or the marrow, whereas Böttcher^{8b} observed in the same material (rat tail), and Siegmund^{10b} in man, essentially the same changes as did Rischpler^{22d} in the mouse tail.

Mechanism of Development of Gangrene.—It has been clearly established that wet cold is distinctly more injurious than dry cold and, further, that immobility, gravity, air currents, interference with the venous return, trauma, debility, localized pressure and antecedent peripheral vascular disease are all factors predisposing to a more severe form of the disease.⁴² Brahdy,⁴³ in a study of frostbite in New York, was not convinced that humidity was a factor in the severity of cold injury, but, since most of his patients were exposed at below freezing temperatures, moisture could have had little effect. Recently, in recognition of the well known variation in susceptibility of individuals to wet cold, Silverman^{2b} and Osborne and Cowen^{28a} have suggested that the person with a labile vasomotor system is more likely to have trench foot than more normal persons.

39. Ungley and Blackwood.^{3b} Böttcher.^{8b}

40. Patterson and Anderson.^{3d} Friedman.^{3e} Siegmund.^{10b}

41. (a) Ribbert, H.: Deutsche med. Wchnschr. 35:2036, 1909. (b) Rischpler.^{22d} (c) von Manteuffel.^{22c}

42. (a) Bigelow, W. J.: Canad. M. A. J. 47:529, 1942. (b) Greene, R.: Lancet 1:303, 1940. (c) Osler, W.: ibid. 2:1368, 1915. (d) Block.¹ (e) Patterson and Anderson. ^{3d} (f) Friedman.^{3e} (g) Böttcher.^{8b} (h) Smith and others.^{23c} (i) Greene.^{24.}

43. Brahdy, L.: J. A. M. A. 104:529, 1933.

Prolonged severe depression of the temperature to -6°C . (21.2°F .) exerts a direct lethal effect on the tissues,⁴⁴ but that is hardly applicable to the problem of trench foot or immersion foot. Therefore, von Mantouffels^{22c} belief that injury due to wet cold is a result of the direct effect of cold is not pertinent to trench foot. The spotty, discontinuous nature of the degenerative process noted in this study and by Friedman^{3e} and Siegmund^{10b} militates against the probability of an external physical agent exerting its effect on the deeper tissues. The genesis of trench foot is somewhat more complicated.

The early physiologic responses to the degrees of depression of temperature in trench foot and immersion foot have been most carefully studied by the London school under Sir Thomas Lewis,⁴⁵ who have clearly shown that, besides the well recognized vasoconstriction, reactive vasodilation may occur, even while the part is exposed to lowered environmental temperature, and that this vasodilation invariably occurs after the part has been warmed. They have further shown that these primary effects are probably mediated through an axon reflex and are related to the release of an "H" or histamine-like substance and that the sympathetic fibers do not participate in the reaction at any phase. These results have in general been corroborated by Hertzman and Roth,^{32a,b} who also have shown that the vasodilator phase is not due to paralysis of the sympathetic vasoconstrictor phase and that the arteries and arterioles are capable of constricting even during the reactive vasodilation phase. The observations of Denny-Brown and associates^{23b} that the sympathetic nerve fibers are the most resistant of all nerve fibers indicate that the nerve fibers responsible for transmitting vasoconstrictor impulses are probably intact in experiments simulating trench foot.

Regardless of their ability to contract in trench foot, for some reason the vessels do not contract, and reactive vasodilation with edema and transudation is clinically⁴⁶ and microscopically⁴⁷ demonstrable. Fell and Hanselman,^{46a} in particular, demonstrated that the transudate may be

44. Lake, N. C.: *Lancet* 2:557, 1917.

45. Grant,^{32c} Grant and Bland,^{33b} Lewis,^{33c,d,e}

46. (a) Fell, E., and Hanselman, R.: *Ann. Surg.* 117:686, 1943. (b) Ungley, C. C.: *Lancet* I:681, 1943. (c) Riehl: *Wien. klin. Wchnschr.* 28:294, 1915. (d) Ungley and Blackwood,^{3b} (e) Lesser,^{3c} (f) Patterson and Anderson,^{3d} (g) Friedman,^{3e} (h) White,^{3h,i} (i) Wieting,³¹ (j) Leriche and Kunlin,^{3a} (k) Stacmmiller,⁹ (l) Berson and Anglucci,^{17c} (m) Large and Heinbecker,^{23a} (n) Davis and others,^{26a} (o) Osborne and Cowen,^{28a} (p) Edwards and others,^{28b} (q) Gruber,^{33a}

47. Friedman,^{3e,f} Hodara,^{8a} Böttcher,^{8b} Kriege,¹² Greene,^{13c} Adami and Nichols,¹⁴ Rischpler,^{22d} Marchand,^{16a} Uschinsky,^{22a} Smith and others,^{23c} Greene,²⁴ Davis and others,^{26a} Gruber,^{33a} Bigelow,^{42a} Greene,^{42b}

extensive enough to affect the dynamics of the circulation. In spite of this obvious evidence, some investigators⁴⁸ have not mentioned the occurrence of a transudate.

Since the discovery of the arteriovenous anastomoses a great deal of work has been done on their anatomy and physiology,⁴⁹ and there can be little doubt of their importance in relation to the control of the temperature and the blood vessels of the extremities. Unfortunately, in spite of the relatively large volume of study, primarily in the form of acute experiments, one must agree with Friedman's^{3e} statement: "A satisfactory detailed anatomic study of the arteriovenous anastomoses in lesions produced by cold has not yet been reported."

When one attempts to correlate the controversial literature on the genesis of the lesions in trench foot and allied diseases, the various theories fall into certain rough categories. One group of investigators is of the opinion that the vascular, epithelial, muscular and connective tissue involvement is secondary to a primary direct effect of the cold on the nerves.⁵⁰ This theory receives some support from the myelin changes that are readily produced in nerves and from the axonal changes produced with more difficulty.⁵¹ However, even if one accepts the still controversial concept that anatomic evidence of nerve destruction precedes that of the vessels, there still exists the possibility that a functional vascular change is primary to the neural changes. Physiologic proof^{32a,b} that practically normal vasoconstrictor impulses are preserved in the presence of marked vascular physiologic reactions would argue against the theory of a primary neural malfunction. It is significant that Denny-Brown and associates^{23b} and Large and Heinbecker,^{23a} who have worked with the finer neuropathologic technics in conjunction with the study of the functional capacity of the nerves, have maintained that the lesions are such as to suggest primary vascular dysfunction.

The adherents of the concept of a primary vascular lesion may be divided into the group advancing the theory of the primary or the sole importance of vasoconstriction⁴⁸ and the majority who regard the succeeding vasodilatation, edema and stasis as most important. The latter

48. Theis.⁷ Nagelsbach.^{17a}

49. Clara, M.: *Ergbn. d. Anat. u. Entwicklungsgesch.* 27:246, 1927. Clark E. R.: *Physiol. Rev.* 18:229, 1938. Clark, E. R., and Clark, E. L.: *Am. J. Anat.* 55:407, 1934. Popoff, N.: *Arch. Path.* 18:295, 1934. Masson, P.: *Bull. Soc. franç. de dermat. et syph. (Réunion dermat., Strasbourg)* 42:117, 1935. Harpuder, K.; Stein, I., and Bycr, J.: *Am. Heart J.* 20:539, 1940. Grant.^{32d} Grant and Bland.^{33b} Lewis.^{33c,e}

50. Blackwood.^{11b} Blackwood and Russell.^{23d,e}

51. Friedman.^{3c} Böttcher.^{8b} Staemmler.⁹ Blackwood.^{11b} Large and Heinbecker.^{23a} Denny-Brown and others.^{23b} Blackwood and Russell.^{23d,e}

in turn may be subdivided into those favoring the absence, or at least the late appearance, of a mechanical vascular obstruction⁵² and those who believe that a vascular obstruction is primary to the whole process.⁵³ As long as there is a mechanical obstruction, it makes little difference whether it is composed purely or partly of erythrocytes. One cannot place much reliance on failure to find thrombi in the reactive zone as proof that they are absent in the acute stages,^{33a} since in persons of the young age group exposed to conditions predisposing to trench foot thrombi are quickly organized. Because of their age, it is improbable that atheromatous plaques were the cause of thrombi in these young soldiers.

The material available in this study when correlated with these divergent views favor the following sequence of events: There is a primary vasoconstriction which coincides with numbness and a pale appearance of the foot; vasoconstriction is then replaced by vasodilation and transudation of fluid; the escape of fluid leaves a dense mass of erythrocytes filling the distended vessel and greatly interfering with the circulation of the blood, a process which gives rise to swelling of the foot, a stage lasting several days. Presumably because of toxic changes in the permeability of the vessel walls, some red cells leak out, causing the formation of hemorrhagic bullae. Thereafter, degenerative changes begin to appear, first functional, later anatomic, in the more specialized structures, epidermis, muscle, nerve and marrow. This last process usually occurred about the time of evacuation and probably was related to the increased vasodilation and hemorrhage occurring when the feet were removed from the cold environment.⁵⁴ Mummification then ensued. Since practically all soldiers in this series received antitetanus immunizations and parenteral treatment with sulfonamide compounds and penicillin, as well as protection from further injury, there was no tendency toward the secondary infection and toxic states described so vividly by Larrey.^{3a} In spite of the obvious structural alterations at this stage, complete functional and pathologic resolution is still possible,² dependent solely on the extent of the vascular obstruction.

52. (a) Winiwarter, F.: *Arch. f. klin. Chir.* 23:202, 1879. (b) Staemmler.⁹ (c) Rischpler.^{22d} (d) Smith and others.^{23c} (e) Gruber.^{33a} (f) Bigelow.^{42a}

53. Block¹ Patterson and Anderson.^{3d} Friedman.^{3e} Hodara.^{8a} Böttcher.^{8b} Siegmund.¹⁰ Kriege¹² Lange and Boyd^{13b} Greene.^{13c} Adami and Nichols.¹⁴ Ziegler.¹⁵ Large and Heinbecker.^{23a} Denny-Brown and others.^{23b} Davis and others.^{26a}

54. Immersion Foot, editorial, *Bull. U. S. Army M. Dept.*, 1943, no. 70, p. 26. White.^{3b,1}

REGENERATIVE ZONE

Regeneration of Epidermis and Sweat Glands.—Fundamentally the reparative ability of the epidermis depends on two factors: first, the inherent capacity of the epithelial cells, whether these originate from the sweat glands or from the surface epidermis, to bridge the defect; second, the presence of a substrate of connective tissue offering circumstances favorable to the realization of this latent regenerative capacity of the epithelial cells. In an exhaustive study of the various factors, Bishop⁵⁵ emphasized the role of the connective tissue substrate as the usual limiting factor. It seems clear that cold, or the degeneration due to cold, is a sufficient stimulus to incite mitotic proliferation, formation of giant cells, and hypertrophy of the epithelial cells.⁵⁶ The deep epidermal papillae with numerous mitoses described by Bishop⁵⁵ and by Fuerst^{26c} attest to the intensity at which this proceeds. Fuerst studied epidermal cells that were stimulated by mild degrees of cold and traced the formation of giant cells from epidermal cells and the cells of sweat glands in much the same manner as in the present study. He did not observe the degeneration of these giant cells—really parakeratosis of the epithelium of the sweat glands and ducts.

However, attention has not been directed to the variation in the reaction of the epidermis at different distances from the demarcation zone or to the relation of this varied proliferative activity to the structure of the connective tissue substrate. This accounts for the fact that some observers^{26c} have noted an increase in epidermal papillae; others,⁵⁷ a decrease, and Staemmler,⁹ both an increase and a decrease. The present study indicates that both occur and that the epidermis over the edematous, inflamed papillary tissue is the site of proliferation and that the thin, flat epidermal strip nearest the demarcation zone, resting on a fibrous avascular substrate, is pushed along passively. This is clearly in agreement with Bishop's conception of the connective tissue substrate.⁵⁵

The tendency of the epidermal cells to grow out along a viable substrate accounts for the observation that the epidermis grows under the gangrenous tissue, adhering to the still viable connective tissue.⁵⁸ This phenomenon would ultimately result in spontaneous amputation and, if the connective tissue substrate is adequate, rec epithelization of the defect. The remarkable ability of the epidermal cells of this age group

55. Bishop, G.: *Am. J. Anat.* **76**:153, 1945.

56. Hodara.^{8a} Rischpler.^{22d} Uschinsky.^{22a} Smith and others.^{23c} Ditt-
rich.^{26b} Fuerst.^{26c}

57. (a) Boland, F.; Claiborne, T., and Parker, F.: *Surgery* **17**:564, 1945.
(b) White and Warren.^{3k} (c) Hodara.^{8a} (d) Siegmund.^{10b} (e) Rischpler.^{22d}
Davis and others.^{26a}

58. Hodara.^{8a} Rischpler.^{22d} Uschinsky.^{22a}

to regenerate is further proved by the observation that within a few months the epidermis of patients with trench foot who have lost no tissue but have had some gangrene is normal.⁵⁹ White and Warren,^{3k} on the other hand, in a study made without the use of normal controls, have reported minimal residual changes such as the flattening of the epidermal papillae.

The interrelationships of epithelial cells, on one hand, and the connective tissue and reticular fibers, on the other, have been studied in the embryo in great detail by Alfejew,⁶⁰ in the adult by Plenck⁶¹ and in tissue cultures by Maximow.⁶² From their observations it is evident that except in early embryonic life the connective tissue forms a basement membrane at all epithelial tissue—connective tissue interfaces, thus separating these two tissues. In the embryo, as the various epithelial invaginations and evaginations occur, this reticular fiber basement membrane is constantly forming, or reticular fibers already present are condensed into a basement membrane separating the epithelial and connective tissue elements. Alfejew⁶⁰ and Plenck⁶¹ have stated that silver impregnation is needed to demonstrate these fibers. The same fundamentals hold true in trench foot, since at no time was there any epithelial regeneration without the presence of a normal basement membrane, demonstrable by silver impregnation, separating the epidermis and sweat glands from the connective tissue substrate, even in highly hyperplastic areas.

Nerves.—The earliest response to the initial damage (myelin breakdown and axonal damage) is an inflammatory reaction with phagocytosis of the myelin.⁶³ This is closely paralleled by Schwann cell proliferation.⁶⁴ The initial inflammation is followed by one of two processes, clearing away of debris and complete regeneration, or marked fibrosis of the nerves, dependent on the intensity of the original injury. In the milder involvements the former was usual⁶⁵; in the more severe (amputation cases) the latter,⁶⁶ but even then numerous nerves, appeared normal. Probably whatever regeneration occurs is complete within a few months, and those nerves that were too damaged have become fibrotic.

59. (a) Paddock, F.: *New England J. Med.* **234**:433, 1946. (b) Block.¹

60. Alfejew, S.: *Folia haemat.* **30**:111, 1924.

61. Plenck, H.: *Ergebn. d. Anat. u. Entwicklungsgesch.* **27**:302, 1927.

62. Maximow, A.: *Ztschr. f. mikr.-anat. Forsch.* **17**:625, 1929.

63. Friedman.^{3c} Staemmler.^{9a} Uschinsky.^{22a} Denny-Brown and others.^{23b} Blackwood and Russell.^{23d}

64. Siegmund.^{10b} Rischpler.^{22d}

65. Block.¹ Denny-Brown and others.^{23b} Smith and others.^{23c} Boland and others.^{57a}

66. Friedman.^{3c} Siegmund.^{10b}

A serious problem is not whether there is anatomic evidence of injury of nerves, since this must be the case where extreme fibrosis has occurred, but how much residual damage of function results. Denny-Brown and associates^{23b} have shown that where nerve regeneration has occurred without fibrosis, normal nerve function tends to occur. Unfortunately, injury was not severe enough in his experiments to result in such marked fibrosis as is seen in amputated specimens of trench foot. But within a short distance above the amputation line there is no evidence of fibrosis, and presumably these nerves would be capable of normal function.¹

Vessels.—In general there are only two mechanisms of vascular repair, recanalization of the preexisting vessel and generation of a new vessel. The details of the reparative process, as well as the pertinent literature, have been summarized by Friedman.^{3e} What has not been previously emphasized is that even in amputation specimens a large number of vessels seem to be anatomically normal^{10b} and that a few centimeters proximal to the demarcation zone vascular lesions are not seen.⁶⁷

The whole process of organization and recanalization of the arteries and the veins is essentially similar to that observed in other vascular occlusions of varied causes.⁶⁸ The intimal proliferation, regardless of whether one believes it to be primary or, which is more probable, secondary to a thrombus, is noninflammatory in nature except immediately adjacent to the demarcation zone.⁶⁹ This granulation tissue organizing the thrombus originates largely inside the internal elastic membrane.^{3e} The minimal changes seen in the arterial media tend to support this thesis. Friedman,^{3e,f} Staemmler^{9a} and Winiwarter^{52a} have pointed out that the endophlebitis begins primarily about the valves. Unfortunately, in the present study the process was too far advanced for one to verify that statement, but the extreme labyrinthine appearance of the lumens seen inside the recanalized veins would tend to support their point of view.

The early development of the collagenous and reticular fibers of the granulation tissue has not been appreciated except by Wertheman.^{68a} But Staemmler^{9a} has commented on the absence of elastic fibers in the newly developed, organizing granulation tissue. In contradiction to Gruber,^{33a} Nagelsbach,^{17a} von Manteuffel,^{22c} and Hellmuth,^{8c} the development of new layers of internal elastic membrane was not noted in this study. The patients in whom this finding is reported to have been made were old men, and so the proliferation of elastic fibers was prob-

67. Block.¹ Boland and others.^{57a}

68. (a) Wertheman, H.: *Virchows Arch. f. path. Anat.* **270**:605, 1928. (b) von Manteuffel.^{22c}

69. Block.¹ Friedman.^{3e} Siegmund.¹⁰ Ducuing and others.^{11a} Gruber.^{33a}

ably related to the normal aging process.⁷⁰ Winiwarter,⁵² who was fortunate enough to study a patient with an eight-year history, described the development of a new internal elastic membrane as demonstrated in the vessels developing in the intima. Judging by the absence of any other reference to this in the literature, one concludes that in the recanalization of the vessels the development of elastic fibers must be a tardy process as opposed to the immediate formation of collagenous and reticular fibers.

The process by which smooth muscle cells are formed from the perivascular fibroblasts has long been known^{65a} and is identical with that by which the smooth muscle cells of the vessels of the embryo and of rabbit ear chambers⁷¹ are formed.

It is doubtful if the recanalized vessels are normal physiologically. The marked narrowing of the lumen, the immature appearance of the new smooth muscle cells and the lack of elastic tissue speak against a normal function. But within a short distance proximal to the amputation line normal vessels are found,⁵⁰ and even in the amputation specimens many of the vessels seemed anatomically normal. From a purely clinical point of view in this series there must have been adequate circulation of the blood by the time amputation was performed, since in most cases it was done just proximal to the demarcation zone and in some cases pinch grafts were successfully applied to bridge epithelial defects at the distal ends of the stumps.

Connective Tissue Proper.—The typical changes occurring in connective tissue in the late stages of trench foot have been described as edema, moderate mononuclear infiltration, absence of polymorphonuclears, phagocytosis of hemosiderin, serous atrophy of fat, and sclerosis.⁷² Edema and mononuclear infiltration, especially the former, are most likely to be found, even in the presence of the mildest type of reaction.

Probably too much attention has been paid to the sclerosis. It is not diffuse but, as pointed out by Friedman,^{3e} is patchy, occurring in islands between the neurovascular septums. Characteristically, the inflammatory cells are lymphocytes and monocytes and various mononuclears derived from these cells. In 1 case in this series and 1 case in Friedman's series^{3e} plasma cells were found, although in other respects these cases seemed not to differ from the usual.

70. Hellmuth.^{8c} Nagelsbach.^{17a} von Manteuffel.^{22c}

71. Clark, E. R.; Hirschler, W. J.; Kirby-Smith, H. T.; Rex, R. O., and Smith, J. H.: *Anat. Rec.* 50:129, 1931.

72. Lesser.^{3c} Friedman.^{3e,f} Hellmuth.^{8c} Blackwood.^{11b} Hecht.^{13a} Nagelsbach.^{17a} Gruber.^{33a} Paddock.^{59a}

Studies of milder forms of trench foot⁷³ have demonstrated that pathologic changes, as seen in biopsy, are slight or nonexistent. In a previous study¹ it has been shown that the connective tissues a few centimeters proximal to the demarcation zone are normal. Even in the amputated tissues, areas with only minimal changes were seen, and one of the purposes of this study is to call to notice the striking tendency toward healing exhibited by the connective tissues in this age group. The angiomatous areas of new vessel formation may also be considered a manifestation of the intense regenerative efforts in this age group.

Except for the observations made by Siegmund,¹⁰ little attention has been paid to the elastic fibers in the connective tissue. The irregularity of elastic tissue is undoubtedly related to the toughness of the tissue and the difficulties of handling the tissues experienced by surgeons operating on these patients. In any event, in trench foot the elastic fibers regenerate much more slowly than any other component.

The lesions of connective tissue seen in chronic pernio bear a startling resemblance to those seen in the regenerative areas of trench foot (McGovern and Wright;⁷⁴ Dittrich^{26b}). It is hard to understand why in trench foot there is an intense tendency toward healing while in pernio there is a marked tendency toward chronicity. The difference is not one of age, since pernio occurring in the younger age group was described by McGovern and Wright. In general the pathology corresponding to the other clinical syndromes due to exposure to wet cold is similar to that of trench foot.

Muscle Regeneration.—Little may be added to the original description of Blackwood^{11b} as far as the histologic appearance of the striated muscle is concerned. He illustrated the residual small atrophic muscle fibers, the marked intermuscular edema and inflammation, the hypernucleated muscle fibers and the loss of cross striation. His findings have been corroborated⁷⁵ in all essentials. White and Warren^{3k} found in biopsy material that, of all the pathologic phenomena described, only the fibrosis and atrophy of individual fibers were present in mild forms of immersion foot without loss of tissue about four months after exposure. In a carefully written, well illustrated paper, Blackwood and Russell^{23e} showed that in the rat at the end of one year the fine terminal myelinated fibers and motor end plates were still slightly abnormal and advanced the thesis that the failure of normal reinnervation is the main cause of the atrophy of striated muscle in the parts exposed to cold.

73. Block.¹ Boland and others.^{57a} Paddock.^{59a}

74. McGovern, T., and Wright, I.: *Am. Heart J.* **22**:583, 1941.

75. Patterson and Anderson.^{3d} Friedman.^{3e} Böttcher.^{8b} Staemmler.^{9a} Siegmund.^{10b} Blackwood and Russell.^{23e}

Bone and Marrow Regeneration.—The marrow and bone have been studied most extensively by Siegmund¹⁰ and Blackwood.^{11b} They have described serous-fatty atrophy and a subacute type of inflammation. Blackwood has maintained that the old bone is resorbed in the early stages and that new bone is laid down after four months. However; the present investigation, as well as most others,⁷⁶ has clearly demonstrated the new, perhaps still uncalcified bone laid down in apposition to the old, dead bone. Without the use of specific calcium impregnations it was impossible to determine whether this new bone was calcified normally or remained as osteoid tissue for an unusually long time.

Friedman^{3e} also noted the phagocytosis of lipid material but did not discuss the source of the phagocytes. Probably they are derived from both the fixed reticular cells and from the mononuclear inflammatory cells (lymphocytes and monocytes).

The fact that fibrosis was restricted to areas near the gangrenous marrow demonstrates that fibrosis is not a universal sequel to tissue severely damaged by cold. In the more aplastic gelatinous areas even the special fiber preparations failed to reveal any increase in collagenous or reticular fibers.

The aplastic marrow, with gelatinous atrophy, mononuclear infiltration, absence of hemopoiesis, prominent reticular cells and dilated vessels, resembled marrow that is in an aplastic stage after irradiation⁷⁷ or after nitrogen mustard therapy.⁷⁸ However, it lacked the large amount of phagocytosed iron pigment seen after employment of these therapeutic agents.

The aplastic area also bore a certain resemblance to embryonic marrow as this appears during the transition from the primary to the secondary marrow described by Maximow.⁷⁹ Especially noteworthy in both instances are the small dark myelocytes, not derived from hemocytoblasts but from histioid wandering cells in the adult. The presence of myelocytes and the absence of erythroblasts are similar to what is seen in the early secondary stage of embryonic marrow.

Analogous to what has been observed after irradiation of marrow and after nitrogen mustard therapy, the whole process in the marrow may be reversible in that the reticular cells would probably be able to serve as

76. Friedman.^{3e} Böttcher.^{8b} Siegmund.^{10b} von Manteuffel.^{22c} Ribbert.^{41a}

77. Bloom, W.: *The Histopathology of Irradiation from External and Internal Sources*, National Nuclear Energy Series, New York, McGraw-Hill Book Company, Inc., 1948.

78. Block, M.; Spurr, C., and Jacobson, L.: *Ann. J. Clin. Path.*, to be published.

79. Maximow A.: *Arch. f. mikr. Anat.* **73**:444, 1909.

a source of heteroplastic hemopoiesis. However, one must remember that the marrow in the extremities is usually quite inactive, probably, as Huggins and Blacksom⁸⁰ showed in the rat tail, because of the lowered temperature with respect to the rest of the body. Therefore the marrow in these amputated toes was more abnormal because of the myxomatous atrophy than because of a decrease in hemopoiesis.

SUMMARY

Three clear zones of tissue change were usually present in trench foot: gangrene, demarcation and reaction. The gangrenous zone was made up of the tissue which had died and then become mummified early after exposure. Because of the mummification, the tissue in this zone was preserved more or less as it had been at the time of tissue death. The major manifestation of injury and probably the most important cause of the gangrene was the spectacular dilation and occlusion of vessels occurring during the phase of reactive dilation leading to infarction of the more peripheral areas. The essential structure—in particular, the fiber scaffolding of the tissues—was remarkably well preserved in this zone.

The gangrenous and the demarcation zone were essentially non-specific in nature. They were similar to comparable zones found in any of the bland ischemic necroses. The only special feature was the intensity and speed with which the reparative efforts took place in the reaction zone, presumably due to the youth and the general good health of the patients, as well as to the lack of any antecedent peripheral vascular disease.

The cellular structure of the corium, especially that of the subpapillary layer, was the most important factor governing the epithelization of any defect. A great deal of normal tissue was always present in the reactive zone, especially near the amputation line. Mild edema, slight subacute inflammation, recanalization of vessels, neoformation of vessels and focal hyalinization were the most common findings in this zone. The reticular and collagenous fibers were laid down very early in the reaction to injury, but at the time of amputation, several months after the initial injury, there was still little evidence of comparable regeneration of elastic fibers. Whatever functional impairment was still present in the

80. Huggins, C., and Blacksom, B.: *J. Exper. Med.* 64:253, 1936.

reactive zone immediately proximal to the gangrenous tissues was probably related to the residual vascular abnormality, absence of elastic fibers and spotty neural fibrosis. However, within few centimeters proximal to the gangrenous tissue there was no evidence of any residual lesions.

Attention is directed to the present complete ignorance of the pathologic anatomy of the glomus in trench foot and related diseases. Perhaps a study of this structure in both warm-blooded and cold-blooded animals which normally live in cold wet weather without apparent disability, would add to the knowledge of this structure and consequently to knowledge of reaction to cold in general.

DEVELOPMENT OF A STATE REFRACTORY TO GROWTH OF A MOUSE TUMOR IMPLANTED IN THE ANTERIOR CHAMBER OF THE GUINEA PIG EYE

JOHN A. SCHILLING, M.D.

AND

ALBERT C. SNELL JR., M.D.
ROCHESTER, N. Y.

IN THE COURSE of studies of heterologous and homologous tissues transplanted into the anterior chambers of guinea pig eyes, it was observed that when growth occurred it was followed almost invariably by regression. In the case of a mouse carcinoma, MT8, it was further noted that after this tumor had grown and regressed in one eye of a guinea pig, a relatively refractory state developed. Only rarely was the same tumor successfully transplanted a second time into the same eye or into the opposite eye. The purpose of this report is to record our observations of the development of this refractory state.

The growth of tissues transplanted from alien species to the anterior chamber of the eye described by Greene¹ suggests that this location provides a more favorable environment for heterologous transplants than does the subcutaneous tissue. However, Greene observed that regression is a common, but not invariable, fate of successful intraocular heterologous tumor transplants. Greene² also observed that regression of a transplant in one eye may be associated with the development of a state relatively refractory toward the same tumor and sometimes another tumor when these are later transplanted into the opposite eye.

Appel and co-workers³ studied the relative degrees to which the ocular and the subcutaneous routes resist transplantation of the Brown-Pearce carcinoma in rabbits. They observed that after a subcutaneous growth of this tumor had become established, the animals were resistant to growth of the same tumor when it was retransplanted subcutaneously but were not resistant to growth of intraocular transplants. These authors

From the Department of Surgery, Division of Cancer Research, University of Rochester School of Medicine and Dentistry.

1. Greene, H. S. N.: *J. Exper. Med.* **73**:461, 1941.

2. Greene, H. S. N.: *Cancer Research* **2**:669, 1942.

3. Appel, M.; Saphir, O.; Janota, M., and Strauss, A. A.: *Cancer Research* **2**:576, 1942.

demonstrated a complement-fixing antibody in the blood scrums of rabbits which were immune to this tumor. The primary aqueous humor of the immunized animals did not contain the antibody, although the secondary or reformed aqueous humor contained it, in variable amounts.

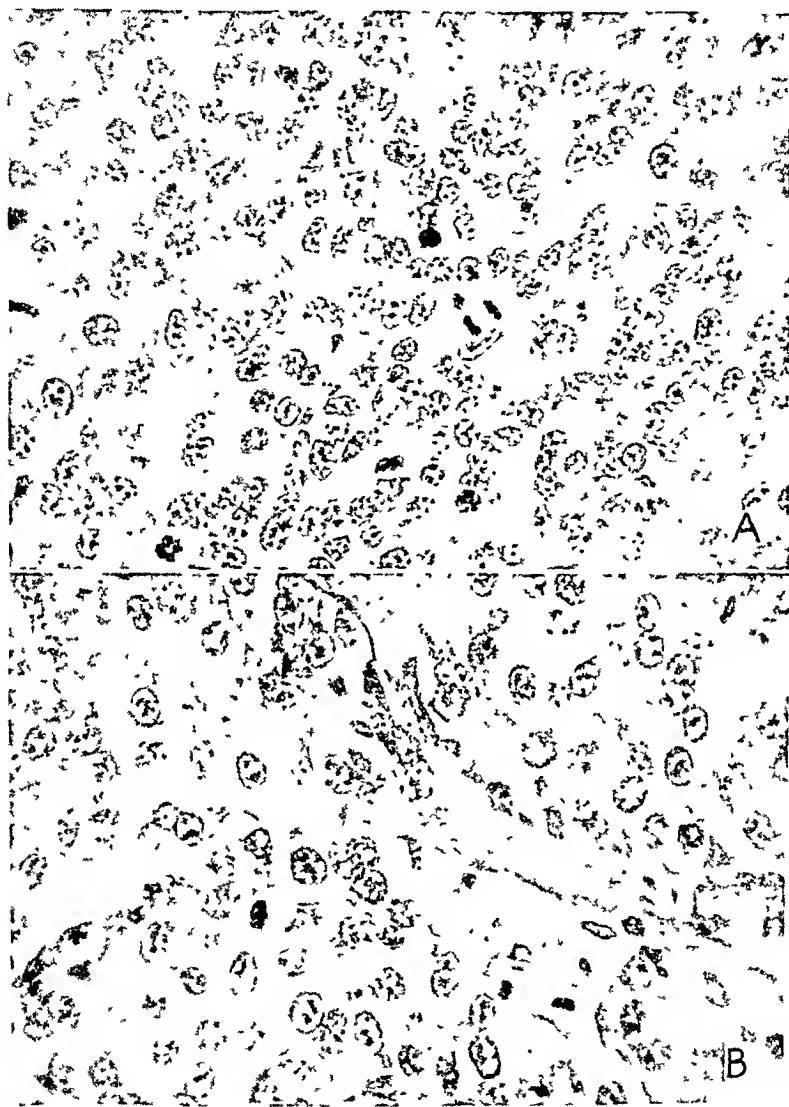


Fig. 1 —A, histologic characteristics of mouse carcinoma MT8 before it was transplanted into the anterior chamber of the guinea pig eye.

B, histologic characteristics of the carcinoma after it had been transplanted into the anterior chamber of the guinea pig eye.

Besredka and Bardach,⁴ however, failed to obtain growth of intra-ocular transplants of the Brown-Pearce carcinoma in rabbits previously immunized against this tumor.

4. Besredka, A., and Bardach, M.: *Compt. rend. Acad. d. sc.* 202:2193, 1936.

Cheever and Morgan⁵ likewise observed that rabbits immunized by any of several routes against the Brown-Pearce carcinoma were refractory to intraocular growth of this tumor. The growing of the tumor in one eye, however, failed to produce a refractory state in the opposite eye.

MATERIALS AND METHODS

For our studies of the regression of intraocular heterologous tumor transplants, we made use of a transplantable bronchogenic mouse carcinoma, MT8, which arose spontaneously in Gardner's laboratory. This tumor was furnished to us by Dr. H. S. M. Greene. The histologic characteristics of this neoplasm before and after it was transferred into the anterior chamber are shown in figure 1. The clinical appearance of the growth in the anterior chamber is shown in

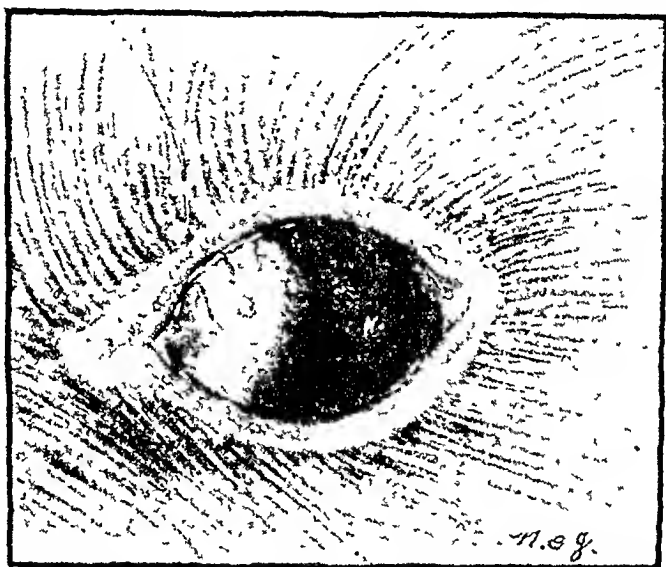


Fig. 2.—Gross appearance of a growing anterior chamber MT8 transplant three weeks after transplantation.

figure 2. The mouse tumor was carried by regular transplantation in the subcutaneous tissue of mice of various strains. The technic of transplantation, although modified in detail, is similar to that described by Greene. Young guinea pigs of both sexes weighing approximately 200 Gm. were used. Anesthesia was induced with an intraperitoneal injection of pentobarbital sodium (6 mg.), supplemented by topical anesthesia of the cornea and the conjunctiva with 2 per cent butacaine sulfate solution. Fragments of tissue 1 to 2 mm. in diameter were removed aseptically from non-necrotic portions of the tumor. The bits of tumor were kept moist in Tyrodes's solution, and representative specimens were set apart for histologic examination. An opening about 3 mm. long was made with a sharp keratome through the cornea into the anterior chamber at the dorsal limbus. A single fragment of tumor tissue was introduced through the incision with a trocar containing a fitted plunger. The tumor fragment was moved to the opposite angle of the anterior chamber by gentle strokes made over the cornea with a spatula. Asepsis was maintained throughout the entire procedure. The eyes were

5. Cheever, F. S., and Morgan, H. R.: *Cancer Research* 2:675, 1942.

examined at suitable intervals with a slit lamp and a corneal microscope. The implant was recorded as growing only when it had enlarged at least threefold and had become vascularized. Care must be taken lest corneal vascularization over the implant be mistaken for vascularization of the implant itself. In other experiments the characteristic appearance of enlargement and vascularization of the implant has never been produced by any reaction other than actual growth..

This particular tumor, unlike some other tumors that we have transplanted successfully, consistently produced an ocular inflammatory reaction characterized by a peripheral corneal vascular pannus and uniform clouding of the cornea. The reaction reached its height about the third day and subsided without residua within a week. When growth occurred, it became perceptible between seven and fourteen days. With this tumor, growth was often slight, the anterior chamber being only one quarter or one third filled. In a few instances the growth nearly filled the anterior chamber, and the cornea appeared to be distended, but rupture of the globe never occurred. Involvement of the vitreous body never was observed, and all instances of growth terminated in regression.

EXPERIMENTS

Three separate series of guinea pigs were studied. Each of 17 guinea pigs comprising the first series received a tumor transplant into the anterior chamber of the right eye. In 9 the transplants grew, and in 8 they were absorbed. Later fragments of the same tumor were transplanted into both the right and the left anterior chamber of each of these animals. The tumor grew in both the left and the right eye of only 1 animal. It grew in the left eyes of 2 other animals.

A second series, 6 guinea pigs, was treated in a similar fashion. Growth occurred in the transplants in the right eyes of 4 of the 6 animals. When the tumor was retransplanted into both eyes of each of the 6 animals, growth did not occur in either eye in a single instance.

A third series, 19 guinea pigs, was then used in a like manner. In 8 animals growth occurred in the right eye, and no growth occurred in the remaining 11. Later, when the mouse tumor was transplanted into both eyes, it failed to grow in any of the animals.

Each time that fresh samples of tumor were used their growth potentialities were tested in control animals. Growth occurred in 20 of 31 of these animals. When the tumor was transplanted simultaneously into both eyes of the control animals, growth occurred in both eyes of 8 of 12 animals used.

Three of the guinea pigs which had become refractory to the growth of MT8 in the manner described received subsequently transplants of a human carcinoma which had been maintained through three guinea pig anterior chamber generations. In 2 of the 3 animals the transplantation was successful. In a fourth animal, which had proved resistant to growth of MT8 on two transfers, the human carcinoma was successfully transplanted into the anterior chamber of the eye.

The usual ocular reaction following the primary transplantation of this tumor was absent on the second transplantation in the right eye but was present and only slightly diminished in the left eye. This observation was quite consistent in all of the animals in which retransplantations were made (fig. 3).

The three series can be described in summary as follows: Forty-two guinea pigs received transplants of MT8 in the anterior chamber of the right eye. No growth was observed in 21 of these animals, while in the other 21 growth of the transplanted tumor was observed. In all instances of growth the tumor ultimately

underwent complete regression. After regression was complete, both eyes of each of the 42 animals received new transplants of the same tumor. No growth occurred in either eye of any of the 21 animals which had failed to show growth on the first transplantation. In the 21 in which growth of the initial transplant, in the right eye, had occurred the second transplants, in both eyes, gave rise to growth in both eyes of 1 animal only and in the left eye only of 2 other ani-

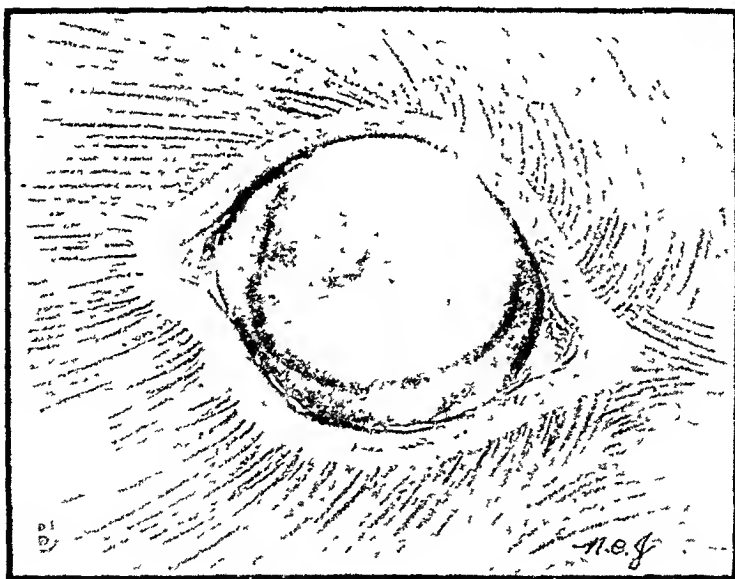
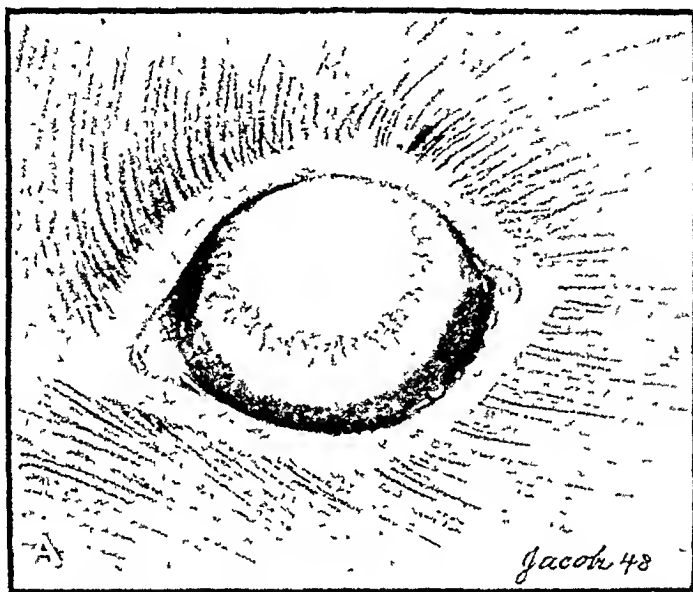


Fig. 3.—A, characteristic appearance of the ocular inflammatory reaction evoked by the initial transplant of MT8 in the guinea pig eye as seen on the fourth day after transfer of the tumor fragment. The transplant is obscured by the clouded cornea.

B, characteristic appearance of a guinea pig eye showing a minimal inflammatory reaction to a second transplant of MT8 on the fourth day after transfer of the tumor fragment. The cornea is relatively clear, allowing visualization of the transplant at the inferior angle of the anterior chamber.

mals. The usual ocular inflammatory reaction was absent in the right eyes and diminished in the left eyes on the second transplantations. The time relationships of growth, regression and retransplantations are summarized in figures 4 and 5.

COMMENT

The experiments described tend to confirm the observations of Greene that a state refractory toward intraocular heterologous tumor transplants is produced in response to a growing (or to a regressing?)

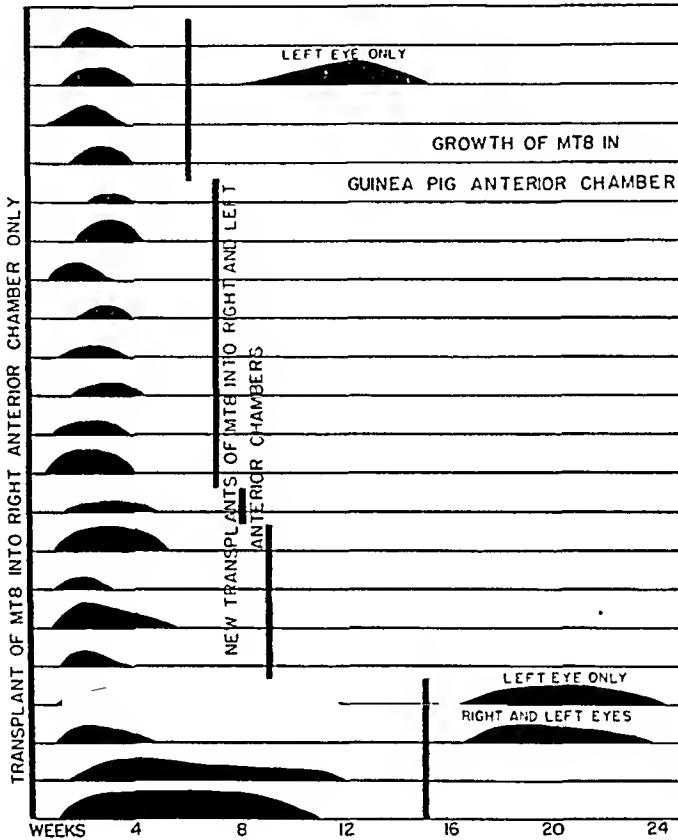


Fig. 4.—Extent and duration of growth of the carcinoma MT8 in initial and secondary transplantations in the guinea pig eye. The blackened area represents the degree of filling of the anterior chamber as well as the duration of growth.

transplant of the same tumor. A monocular transplant confers the refractory state on both eyes. The degree of specificity of this effect has not been studied closely. It was noted, however, that the refractory state applied to the same tumor whether the tumor was carried in C3H or dba strains of mice. Also, 3 of 4 guinea pigs refractory toward growth of MT8 mouse carcinoma were not refractory to growth of intraocular transplants of a human carcinoma that had previously been carried through three generations of anterior chamber transplants.

It is noteworthy that those animals which did not tolerate growth of the first transplants were never subsequently inoculated successfully with the same tumor. (One of these animals tolerated growth of a different tumor.) In other words, half of the experimental animals appeared to have a natural resistance which prevented growth of the transplants. Other animals tolerated growth of the transplants only to a slight and brief extent, while still others behaved as if effective resistance developed slowly and weakly. These differences could be explained by the postulate that the ocular resistance varies among different individuals of the same species. (Uniformity of the tumor samples trans-

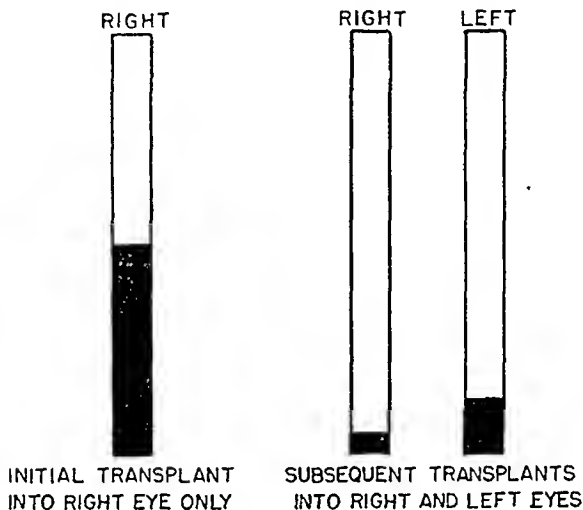


Fig. 5.—Proportions of growth on initial and subsequent transplantations of the carcinoma MT8 in the anterior chamber of the guinea pig eye. The blackened area represents the incidence of successful transfer.

planted was closely checked.) The experimental results indicate that weakly resistant individuals become more effectively resistant after contact with the tumor. Alterations in the reactive state of the eye are further evidenced by the variations observed in the general inflammatory reactions of the part of the cornea and the conjunctiva between initial and subsequent transplants of the same tumor.

The unique receptiveness of the eye to various types of tissue transplants can be described by the generalization that the eye reacts against such transplants relatively weakly or sluggishly. This does not indicate whether the reactions of the eye differ in kind or merely in degree and/or rate from the reactions of other sites. Nevertheless, the relatively protracted and leisurely course of the ocular reactions, coupled with the convenience with which these reactions may be observed through the cornea, affords certain advantages in the study of the fate of hetero-transplants.

The individual variations of susceptibility to transplantations and the alterations of susceptibility and of the inflammatory reactions observed after the cornea has been in contact with the tumor provide a basis for suspecting that something in the nature of immune reactions occurs in the case of intraocular heterotransplants. If this is so, immune type reactions could be responsible for the frequently observed phenomenon of regression of temporarily flourishing intraocular heterotransplants.

SUMMARY

After transplants of a mouse tumor, MT8, had temporarily flourished in the anterior chambers of guinea pig eyes and then regressed, a relatively refractory state developed, evidenced by the fact that subsequent transplantations were only rarely successful. Monocular transplants conferred the refractory state on both eyes. The possibility that this may indicate an immune type of reaction is considered.

PARADOXIC EMBOLISM

A Review of the Literature, with Report of a Case in Which This Condition
Followed the Administration of "Dicumarol"

RAYMOND L. YOUNG, M.D.

R. C. DERBYSHIRE, M.D.,

AND

O. S. CRAMER, M.D.

ALBUQUERQUE, N. MEX.

PARADOXIC EMBOLISM is defined as embolism in which the embolus arises from a vein but lodges in a systemic artery instead of in the pulmonary artery. To accomplish this, it must pass through a septal defect in the heart, which is usually a patent foramen ovale.

According to Thompson and Evans,¹ the earliest instance mentioned in the medical literature is that reported by Cohn in 1860. But it was not until 1877 that the first detailed report of a case was given, by Cohnheim. He described recurrent embolism of the right middle cerebral artery in a case of widely patent foramen ovale with thrombosis of the veins of the lower extremities. Five years later Zahn was able to demonstrate, in a case of extensive thrombosis of the iliac veins, a long embolus the thickness of a pencil passing through a persistent foramen ovale. A similar observation was made by Hauser in 1888. Von Recklinghausen² first suggested the term "paradoxical embolism" to describe this condition.

Since the earlier reports, a few isolated cases of paradoxical embolism have been reported, the two largest series consisting of 7 cases each (Thompson and Evans¹; Ingham³). The accompanying table gives a summary of the 41 recorded cases. All of these were verified at autopsy except for that of Porter.⁴ In his patient the symptoms and signs were so convincing that a clinical diagnosis could be made with a reasonable degree of certainty, even though the patient recovered. In 33 of the 40 cases confirmed by autopsy, the paradoxical embolism was due to a patent foramen ovale, and in the remainder, to a patent interventricular septum.

From the Departments of Gynecology, Surgery and Internal Medicine of the Lovelace Clinic.

1. Thompson, T., and Evans, W.: *Quart. J. Med.* **23**:135, 1930.
2. von Recklinghausen, cited by Thompson and Evans.¹
3. Ingham, D. W.: *Am. J. M. Sc.* **196**:201, 1938.
4. Porter, A. G.: *Lancet* **2**:634, 1941.

In about 50 per cent of the cases cerebral embolism was the most conspicuous feature of the disease, while in the rest multiple emboli were present.

The making of a diagnosis of paradoxical embolism during life is usually extremely difficult. In the majority of cases the diagnosis has been made only at autopsy. A notable exception to this is the case reported in 1941 by Porter,⁴ already mentioned, in which the patient recovered. According to Porter, such a diagnosis should be seriously considered if in the presence of venous thrombosis followed by pulmonary embolism and recovery there are later signs of infarction in other organs. If the acute attack is preceded by cerebral symptoms, one may consider the diagnosis more positively.

*Cases of Paradoxical Embolism Reported in the Literature**

Author	Year	Cases
Cohn, B.: Klinik des embolischen Gefasskrankheiten, Berlin, A. Hirschwald, 1860.....	1860	1
Cohnhelm, J.: Vorlesungen über allgemeine Pathologie, Berlin, A. Hirschwald, 1877, vol. 1, p. 131.....	1877	1
Louis, cited by Ballet, G.: Arch. gén. de méd. 5:659, 1880.....	?	1
Zahn: Rev. méd. de la Suisse 1:227, 1881.....	1881	1
Rostan: Thésis, Geneva, 1884.....	1884	1
Hauser: München. med. Wchnschr. 35:583, 1888.....	1888	3
Ohm: Ztschr. f. klin. Med. 61:374, 1907.....	1907	1
Hensel, R.: Deutsche med. Wchnschr. 47:625, 1921.....	1921	1
Beattie: Internat. A. M. Museums Bull. 11:64, 1925.....	1925	2
Versé: Verhandl. d. deutsch. path. Gesellsch. 13:215, 1930.....	1930	2
Bernard: Quart. J. Med. 23:305, 1930.....	1930	1
Thompson and Evans ¹	1930	7
French: Arch. Patn. 11:383, 1931.....	1931	1
Taylor: Arch. Path. 16:901, 1933.....	1933	1
Armand-Delille and Lesobre: Bull. Soc. de pédiat. de Paris 33:274, 1935.....	1935	1
Hirschboeck, F. J.: Am. J. M. Sc. 189:236, 1935.....	1935	1
Koritschoner, R.: J.A.M.A. 106:1269, 1936.....	1936	1
Neely: Nebraska M. J. 21:61, 1936.....	1936	1
Jones, R.: Brit. M. J. 2:225, 1936.....	1936	1
Ingham ³	1936	7
Hanna, R.: Am. J. Dis. Child. 62:555, 1941.....	1941	1
Porter ⁴	1941	1
Vintrup, B.: Nord. med. (Hospitalstid.) 10:1839, 1941.....	1941	2
Birch, C. A.: Brit. M. J. 2:727, 1945.....	1945	1

*All but the case of Porter (1941) were confirmed by postmortem examination.

The following case is reported not only because of the interest aroused by the rarity of such a lesion but also because, so far as can be determined, it is the first case in which such a complication has followed the use of an anticoagulant drug.

REPORT OF CASE

A 50 year old white woman, married, first seen on July 29, 1947, complained that there had been profuse vaginal bleeding for the preceding three days, pelvic discomfort for six months and large, painful varicosities of both lower extremities which had become gradually worse during the preceding few years.

The family history was noncontributory. The past history was essentially irrelative except that for many years the patient had suffered recurrent attacks of pain in the right upper quadrant of the abdomen, accompanied by nausea and vomiting, concerning which a diagnosis of disease of the gallbladder had been

made elsewhere. She had had seven uneventful term pregnancies. Her menstrual function had been normal throughout her reproductive life, and she stated that her last normal menstrual period had occurred about one year before her present illness. Specifically, there was no past history suggestive of cardiovascular disease.

She was a well developed, moderately obese white woman about 50 years old, who did not appear to be in acute distress. The skin and the mucous membranes were normal. The heart and the lungs were entirely normal, and the blood pressure 140 systolic and 90 diastolic. Except for the palpation of a hard abdominal mass arising from the lower part of the pelvis and extending 12 cm. above the symphysis, abdominal examination showed no abnormality. The liver was not enlarged, and there was no tenderness in the region of the gallbladder. Pelvic examination confirmed the presence of a uterine tumor of the size described; the adnexa could not be palpated because of obesity. The cervix was patulous and bore old, healed lacerations. The patient was bleeding moderately from the uterine cavity, and the vagina was filled with freshly clotted blood. There were extensive varicosities of both internal saphenous systems.

Urinalysis gave normal results. The red blood cell count was 4,300,000; the hemoglobin content, 86 per cent. The white cell count was 7,800, with a normal differential count. The Kahn test revealed no syphilis.

A preoperative diagnosis of leiomyoma and varicose veins was made. The following day ligation of the saphenous veins was done by one of us (R.C.D.), followed immediately by total hysterectomy, bilateral salpingo-oophorectomy and appendectomy (R.L.Y.). Findings at the time of operation confirmed the presence of a uterine tumor measuring 13 by 10 by 8 cm. A diagnosis of adenomyosis uteri, chronic salpingitis and chronic cystic cervicitis was made. Palpation of the gallbladder did not reveal stones, nor were abnormalities of other abdominal organs found. Bleeding at the time of operation was not excessive or difficult to control, a liberal estimate of 150 cc. total blood loss being made.

Immediately after the operation she was given intravenously 1,000 cc. of an isotonic sodium chloride solution containing 5 per cent dextrose. The postoperative course was uneventful until twenty-four hours after the operation, when she began to bleed profusely from all the incisions—the abdominal, the vaginal and both of the subinguinal incisions—and rapidly entered into profound shock. The blood pressure and the radial pulse were unobtainable; the apical pulse rate was 160 per minute. One hour after the onset of bleeding, the red blood cell count was 2,810,000; the hemoglobin content, 7.8 Gm. (50 per cent); the platelet count 281,000; the coagulation time, 4.5 minutes; the bleeding time, 1 minute; the prothrombin time (Quick), 27 minutes, or less than 1 per cent.

During the next six hours she received 250 cc. of blood plasma, 250 cc. of isotonic sodium chloride solution and 1,500 cc. of freshly citrated whole blood. In addition, 60 mg. of vitamin K was given intravenously every four hours until the prothrombin time returned to normal. Bleeding from the wounds gradually decreased but did not cease completely until twenty-four hours after its onset. The blood pressure first returned to normal eight hours after the onset of the bleeding.

Twenty-four hours later the prothrombin content was 100 per cent, the red blood cell count 2,900,000 and the hemoglobin content 9.0 Gm. The skin for about 8 cm. around the abdominal wound was intensely ecchymotic, and the arms presented ecchymotic areas wherever hypodermic injections had been given. Additional whole blood was given during the next few days until the hemoglobin was 11.0 Gm., and her condition gradually improved. Liver function tests were made to determine the cause of the great increase of prothrombin time. The

sulfobromophthalein sodium test revealed 6.5 per cent retention in forty-five minutes. Roentgenograms of the gallbladder revealed a nonfunctioning organ without evidence of stones.

Because of a persistent low grade daily fever, penicillin was given. An infected hematoma soon developed in the abdominal wound and was evacuated on the fourteenth postoperative day, yielding about 200 cc. of old blood. This continued to drain sanio-purulent material throughout the postoperative course.

Four weeks after the episode of bleeding, when her condition was apparently good, the patient confided that prior to undergoing surgical treatment she had consulted an osteopathic physician, who had given her a week's supply of small white capsules with instructions to take one three times a day "to keep the blood in her varicose veins from clotting." On further investigation the capsules proved to contain 50 mg. of "dicumarol" (3,3'-methylcnebis [4-hydroxycoumarin]) each. It was estimated that she had taken a total of 950 mg. of "dicumarol" during the week preceding the operation. During the administration of this drug, prothrombin times were not determined, and the patient remained ambulatory in another town, having been advised to return for further examination in a week.

Five weeks after the operation she suddenly complained of pain in the left side of her chest. Examination revealed a pleural friction rub over the lower lobe of the left lung posteriorly, and a roentgenogram showed, near the cardiac apex, an area of consolidation which measured about 2 cm. in diameter. A final differentiation between pulmonary embolism and pneumonia was not made, but further anticoagulant drugs were not given. Low grade fever continued despite antibiotic drugs, but the patient had no further complaints.

One week later she suddenly became cyanotic and orthopneic and complained of inability to move the right foot and leg. Shortly thereafter she complained of weakness of the left hand. Both lower extremities were pale, cold and clammy. There was no limitation of voluntary motion of the left leg, though the patient was unable to move the right leg. The grip of the left hand was definitely weaker than that of the right. A diagnosis of saddle embolus was entertained, with possibly a cerebral accident, but because of the infection of the abdominal wall, embolectomy was not attempted. The patient was treated with morphine, papaverine and oxygen. Twelve hours later the right leg became intensely painful, cold and cyanotic. A diagnosis of embolism of the right iliac artery was made, and sympathetic nerve block was undertaken. The patient died while this procedure was being performed.

Autopsy (six hours after death, by T. R. Moran).—A superficial abscess of the fat was present in the operative scar. The liver was not enlarged. The right femoral and common iliac arteries contained an antemortum clot 15 cm. long. The right hypogastric vein was filled with a thrombus. The inferior vena cava contained a hard, dry clot, which extended throughout its length but did not completely fill the lumen. The aorta contained an embolus, which extended from the heart itself throughout the aortic arch. A patent foramen ovale was present which measured 9 mm. in diameter. The semilunar valve was held open by an embolus; the short rounded end of this protruded into the left auricle, while the proximal longer end extended into the right auricle. Both pulmonary arteries contained massive, firm, dry emboli. Both lungs revealed patchy areas of infarction. Other organs were grossly normal. The brain was not examined.

COMMENT

Although patency of the foramen ovale is one of the commonest of all congenital abnormalities, being found in from 20 to 35 per cent of

all autopsies, there are several factors which contribute to the rarity of paradoxical embolism. The first of these is the size of the septal defect. Thompson and Evans¹ found a patent foramen ovale in 386 of 1,100 autopsies (35.1 per cent). In 319 cases (29 per cent) the opening was only large enough to admit a small probe. In only 67 cases (6 per cent) could an ordinary lead pencil be passed through the foramen.

The second factor is the pressure relationship between the auricles. In the majority of cases of patent foramen ovale, the opening on the side of the left auricle is guarded by a valvelike fold of endocardium. Since the pressure in the left auricle is normally higher than that in the right, auricular contraction will insure closure of the defect, thus effecting physiologic competence.

According to Wittig,⁵ however, 50 per cent of all the cases of paradoxical embolism are preceded by pulmonary embolism. This results in a rise of pressure in the right auricle and a decrease in the left, thus allowing blood to flow from right to left through the patent foramen. Before this can occur, however, it is necessary for at least one third of the pulmonary circulation to be occluded. Since occlusion of 50 per cent or more of the pulmonary circulation causes immediate death, it is obvious that many of the patients will die before paradoxical embolism has had time to develop. Furthermore, if a nonfatal infarct has been formed of sufficient size to increase the pressure in the right auricle—thus setting the stage for the development of paradoxical embolism—it is still necessary for a second embolus to reach the right auricle from a peripheral vein. If, as is often the case, the embolus represents a cast of the vessel in which it originates, it is obvious that the foramen ovale must be of rather large size to permit its passage.

In the 50 per cent of the cases in which paradoxical embolism is not associated with pulmonary embolism, the explanation is more difficult. One suggestion is that minute bacterial emboli are able to pass through small defects and lodge in the peripheral arteries.

In our own case a small pulmonary embolus had occurred at least one week before death. While this embolus was hardly of sufficient size to elevate the pressure of blood within the right auricle, the second and more massive pulmonary embolus which was found at autopsy undoubtedly preceded the development of the paradoxical embolus. The septal defect measured 9 mm. in its greatest diameter; once pressure relationships between the auricles were reversed, paradoxical embolism was the almost inevitable sequel.

As in 50 per cent of the recorded cases, clinical signs of cerebral embolism were evident shortly before death. Although examination of the

5. Wittig, M.: *Ztschr. f. Kreislaufforsch.* 19:505, 1927.

brain was not permitted, it can reasonably be assumed that cerebral embolism was present.

As has already been mentioned, this is the first recorded case in which paradoxical embolism has followed the use of an anticoagulant drug. Whether or not its administration was responsible for the death of this patient can, of course, only be postulated. While the danger of thromboembolic disease is always present after a pelvic operation, certain conditions known to predispose to its development were either caused or aggravated by the administration of "dicumarol." Notable among these were the long-continued venous stasis resulting from the profuse post-operative bleeding and the massive, infected hematoma of the abdominal wall.

Despite repeated warnings in the medical literature and the explicit instructions which accompany the product, the improperly controlled use of "dicumarol" continues.⁶ In 1943, Link,⁷ the synthesist of this drug, commented that "the briefest meditation on the strictly theoretical aspects of the clotting phenomenon leaves us with the appalling feeling that tampering with coagulability of the blood . . . is a hazardous business." It is hoped that, as more and more cases of "dicumarol" poisoning are reported, physicians who undertake the responsibility of administering "dicumarol" will listen to Link's warning.

SUMMARY

Forty-one cases of paradoxical embolism have been collected from the literature. These are reviewed, and an additional case, the first to be complicated by "dicumarol" poisoning, is reported.

Factors predisposing to the occurrence of paradoxical embolism are discussed, and the probable mechanism of development is outlined.

The possible influence of the administration of "dicumarol" on the development of paradoxical embolism is discussed, and warning is again made against the improperly controlled use of this drug.

6. Draper, A. J.: J.A.M.A. 136:171, 1948.

7. Link, K. P.: Anticoagulant From Spoiled Sweet Clover Hay, in Harvey Lectures, 1943-1944, Baltimore, Williams & Wilkins Company, 1944, p. 162.

HEREDITARY RENAL DISEASE AND AMYLOIDOSIS IN MICE

W. E. HESTON, Ph.D.

AND

MARGARET K. DERINGER, Ph. D.
BETHESDA, MD.

MOST strain A mice in which tumors of the mammary glands do not develop die of a degenerative disease of the kidney commonly referred to as nephritis. The onset of the condition occurs in approximately 100 per cent of the animals by 12 months of age, and few live much beyond 18 months.

In 1938, Andervont¹ noted the common occurrence of nephritis in this strain, and later Gorer² referred to the condition as cystic disease of the kidneys and described it as a special type of hydronephrosis in which obstruction of the tubules was brought about by focal necrosis occurring at the tip of the renal papilla. A more comprehensive description of the pathologic aspects of the condition has been given by Dunn,³ who related the renal disorder to amyloidosis. She described the course of the disease as beginning with deposition of amyloid between the tubules of the papilla, followed by obstruction of the tubules, which results in destructive changes that radiate to the surface of the kidney.

While the condition has been emphasized in regard to strain A, it also occurs in strain Y. Approximately 100 per cent of the mice of this strain show the condition as early as those of strain A do. In certain other strains, however, the incidence is low. In the study reported herein a low incidence was recorded for strain L. Gorer reported that the disease had been observed in only a few strain C₅₇ black mice and in those at an advanced age. He did not find it in his series of strain CBA. Dunn recorded that but 1 of 150 strain C₃H mice examined was affected.

These strain differences indicate a genetic background for the condition and suggest that hybridization studies would be of interest. F₂ and back-cross ratios could be expected to give some indication of the num-

From the National Cancer Institute, National Institute of Health, United States Public Health Service.

1. Andervont, H. B.: Pub. Health Rep. **53**:232, 1938.
2. Gorer, P. A.: J. Path. & Bact. **50**:25, 1940.
3. Dunn, T. B.: J. Nat. Cancer Inst. **5**:17, 1944.

*Occurrence of Nephritis and Amyloidosis in Strains A, L and Y and Their Hybrids**

Type of Mice	Age in Months																Total	Per Cent	
	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23			24
Strain A mice																			
Number with nephritis			1	3	19	12	4	4	4	7	35							85	91.8
Number with amyloidosis			1	2	14	11	4	4	4	7	35							78	95.3
Number with both			1	3	17	10	4	4	4	7	35							81	88.2
			1	2	13		9	4										75	
Strain L mice																			
Number with nephritis			3(1)	1(0)	1(0)	1	2	4	14(12)	7	83(77)	3	4(3)	10	6	3	25	167(154)	
Number with amyloidosis			2(0)	1(0)	1(0)	0	0	1	1(0)	0	4(3)	1	1(0)	1	1	1	4	19(12)	7.8
Number with both			2(0)	1(0)	1(0)	0	0	0	2(1)	0	6(5)	2	3(2)	1	2	1	10	31(24)	15.6
			2(0)	1(0)	1(0)	0	0	0	1(0)	0	4(3)	1		0			2	15(8)	5.2
LAF ₁ hybrids																			
Number with nephritis										1	42(41)	1	2	4(3)	3	7	77(73)	137(131)	
Number with amyloidosis										0	1(1)	0	0	1(0)	0	0	5(1)	7(2)	1.5
Number with both										1	1(0)	0	0	2(1)	1	5	31(27)	41(35)	26.7
										0	0(0)	0	0	1(0)	0	0	5(1)	6(1)	0.8
ALF ₁ hybrids																			
Number with nephritis		1			3	1		2		1	35	1	3	2	2	3	31(30)	85(84)	
Number with amyloidosis		0		0	0	0		0		0	0	0	0	0	0	1	3(2)	6(5)	6.0
Number with both		0		0	0	0		0		0	3	0	1	2	0	2	28(27)	36(35)	41.7
		0		0	0	0		0		0	0	0	0	0	0	1	3(2)	5(4)	4.8
F ₁ hybrids (combined)																			
Number with nephritis		1			3	1		2		2	77(76)	2	5	6(5)	5	10	108(103)	222(215)	
Number with amyloidosis		0		0	0	0		0		0	1	0	2	1(0)	0	7	8(3)	13(7)	3.3
Number with both		0		0	0	0		0		1	4(3)	0	1	4(3)	1	1	59(54)	77(70)	32.6
		0		0	0	0		0		0	0	0	1	1(0)	0	1	8(3)	11(5)	2.3
LAF ₂ hybrids																			
Number with nephritis		1		2(1)	6(3)	5(0)	5(0)	1	3(2)	2	45(42)	2(1)	2	6(5)	6(5)	1	18(17)	105(83)	
Number with amyloidosis		0		1(0)	2(0)	2(0)	3(0)	1	2(1)	2	23(21)	1	1	2(1)	4(3)	0	4(3)	48(34)	41.0
Number with both		0		1(0)	4(1)	2(0)	5(0)	0	2(1)	2	28(25)	1	1	4(3)	5(4)	0	10(9)	65(47)	56.6
		0		1(0)	2(0)	2(0)	3(0)	0	2(1)	2	23(21)	1	1	2(1)	4(3)	0	3(2)	46(32)	38.6
ALF ₂ hybrids																			
Number with nephritis		1		1	2(0)		1	1(0)	8	1(0)	44(40)	2	4(3)		2(1)	2	17(16)	87(76)	
Number with amyloidosis		0		0	1(0)	1	1	1(0)	4	1(0)	6(3)	0	2(1)		1(0)	1	3(2)	21(12)	15.8
Number with both		0		0	1(0)	1	1	1(0)	5	1(0)	11(8)	1	2(1)		2(1)	1	12(11)	38(29)	38.2
		0		0	1(0)	1	1	1(0)	4	1(0)	5(3)	0	2(1)		1(0)	0	3(2)	19(11)	14.5
F ₂ hybrids (combined)																			
Number with nephritis		1		3(2)	8(3)	5(0)	6(1)	2(1)	11(10)	3(2)	89(82)	4(3)	6(5)	6(5)	8(6)	3	35(33)	192(159)	
Number with amyloidosis		0		1(0)	3(0)	2(0)	4(1)	2(1)	6(5)	3(2)	29(24)	1	3(2)	2(1)	5(3)	1	7(5)	69(46)	28.9
Number with both		0		1(0)	5(1)	2(0)	6(1)	1(0)	7(6)	3(2)	39(33)	2	3(2)	4(3)	7(5)	1	22(20)	103(76)	47.8
		0		1(0)	3(0)	2(0)	4(1)	1(0)	6(5)	3(2)	29(24)	1	3(2)	2(1)	5(3)	0	6(4)	66(43)	27.0

Type of Mice	Age in Months																			Total	Per Cent
	Age in Months																				
	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24				
A back-cross hybrids	1	1	0	2	3(2)	1(0)	0	2(1)	2	5(4)	56(54)	1	4(3)	1(0)	6	1	22(19)	108(97)			
Number with nephritis	0	0	0	0	0(0)	0	0	2(1)	1	5(4)	33(32)	1	4(3)	1(0)	5	1	3(3)	56(51)	52.6		
Number with amyloidosis	0	0	0	1	1(0)	1(0)	0	2(1)	1	5(4)	36(35)	1	3(2)	1(0)	5	1	14(11)	72(62)	63.9		
Number with both	0	0	0	0	0	0	0	2(1)	1	5(4)	32(31)	1	3(2)	1(0)	4	1	3(3)	52(48)	49.5		
L back-cross hybrids		2		4(2)	6		2	6(4)	1	1	27(19)		5(3)	6(4)	1(0)	2	13	76(69)			
Number with nephritis		0		0	0		0	0	0	1	3(0)		1	1(0)	1(0)	0	1	8(3)	4.3		
Number with amyloidosis		0		0	1		2	0	0	0	7(2)		0	2(1)	1(0)	2	10	25(18)	23.7		
Number with both		0		0	0		0	0	0	0	3(0)		0	1(0)	1(0)	0	1	6(1)	1.4		
Strain Y mice	1				3													4	100		
Number with nephritis	1			3	3													4	100		
Number with amyloidosis	0			3	3													3	75		
Number with both	0			3	3													3	75		
AYF ₁ hybrids																					
Number with nephritis								90										90	100		
Number with amyloidosis								90										90	100		
Number with both								90										90	100		

*Numbers of mice in groups after mice with secondary amyloidosis have been excluded are given in parentheses. The percentages relate to the restricted groups.

ber of genetic factors involved, and the segregation of amyloidosis in relation to that of the renal disease could be expected to confirm or deny their relationship and, furthermore, would indicate which condition was more directly the result of the genic action. This paper reports the results of such hybridization studies.

PROCEDURE

The work was concerned principally with an analysis of the difference between the high nephritis strain A and the low nephritis strain L (referred to as "strain C₅₇ leaden" by Law⁴) but observations on high nephritis strain Y and the first generation hybrids between strain Y and strain A were also recorded. All three are highly inbred strains and are of the colonies maintained at the National Cancer Institute. All were derived from the stocks of the Roscoe B. Jackson Memorial Laboratory. The strain A and strain L stocks were obtained in 1938 and the strain Y stock in 1936.

Reciprocal matings were made between the two parent strains A and L for producing the ALF₁ hybrids with strain A mothers and LAF₁ hybrids with strain L mothers. These reciprocal F₁ hybrids were in turn mated inter se to produce ALF₂ and LAF₂ hybrids, and also were mated to the parent strain A to produce A back-cross hybrids and to the parent strain L for L back-cross hybrids. Mice of the two parent strains and of the hybrid groups were segregated as to sex and maintained in wooden cages with 8 mice to the cage except during the time they were in the breeding cages. Then 4 females were mated to 1 male and when pregnant were isolated in separate cages for rearing their litters. All groups were fed Purina dog chow and were given a constant supply of tap water.

At the outset it was planned to kill and examine sample groups at 18 and 24 months of age in order to tabulate the number with amyloidosis and the number with nephritis at these age periods. Some of the animals, however, were found moribund or dead and had to be examined at other age levels. None of the strain A mice were kept beyond 18 months, because at this age their mortality was high. None of the other groups were kept beyond 24 months.

The kidneys of all mice were fixed and sectioned so as to include the papilla to ascertain the presence or the absence of degeneration at this site. After establishing the fact that the amyloidosis was systemic, the routine diagnosis was made on observation of sections of only the spleen and the duodenum. These organs were chosen because the interstitial tissue of Brunner's glands and the spleen have been found to be the two locations in which amyloid is most likely to occur in the mouse. The tissues were fixed in Tellyesniczky's fluid (70 per cent ethyl alcohol, 20 parts; formaldehyde solution U.S.P., 2 parts; glacial acetic acid, 1 part) and were stained with hematoxylin and eosin. The material considered as amyloid had the same localization as that which Dunn³ described when working in this laboratory with the strain A mice, and has been identified by Turnbull,⁵ after using special staining procedures, as mouse amyloid.

To obtain data on a cross between two strains both of which had a high occurrence of amyloidosis and nephritis, sections were made of the kidneys, duodenums and spleens of 4 strain Y males and a sample group of 90 F₁ hybrids resulting from crossing these strain Y males to strain A females. Three of the

4. Law, L. W.: J. Hered., to be published.

5. Turnbull, H. M.: J. Path. & Bact. 57:18, 1945.

strain Y males were killed and examined at 12 months of age and 1 at 8 months, and all of the AYF_1 hybrids were killed and examined at 15 months of age. These animals were a part of an experiment for analyzing the relationship of the lethal yellow gene and spontaneous pulmonary tumors, but had been reared under conditions identical with those described for the other groups.

RESULTS

The rates at which amyloidosis and nephritis occurred in the inbred lines and in the various hybrid groups are recorded in the table. Aside from listing the total number, the number with nephritis and the number with amyloidosis, there is listed also the number with both conditions. From these figures one can readily determine the number with nephritis even though amyloid was not observed, or the number with amyloidosis and not nephritis.

Unfortunately, the picture was somewhat confused by the fact that in certain of the animals secondary amyloidosis was noted—distinguished from the primary condition by the presence of some other chronic disease. For the most part these were animals which, in consequence of being infested by the small mite *Myobia musculi*, had had severe dermatitis for a considerable period. Persistent scratching of the dorsal region of the head had resulted in extensive destruction of tissue; in some mice even the ears and the eyes were eroded. Lymph nodes, particularly those of the cervical region, were often greatly enlarged. In a few other animals large abscesses or other chronic infections suggested the possibility that the amyloidosis could be secondary. Generally in these animals the amyloidosis had a slightly different manifestation in that infiltration occurred earlier and to a greater degree in the spleen than in the duodenum. Nephritis occurred in the mice showing secondary amyloidosis just as in those that showed primary amyloidosis.

For the genetic analysis we eliminated the data concerning mice in which examination showed chronic diseases that could have caused amyloidosis, and determined incidence of amyloidosis and nephritis and segregation ratios only on the restricted data. Both the original and the restricted data are included in the table.

No sex difference was found in the incidence of either amyloidosis or nephritis in any group, and therefore the sexes are not segregated in the final tabulation. In all groups, however, the number of males and the number of females were approximately equal.

The data concerning the strain A mice confirm previous observations that most animals of this strain show both amyloidosis and nephritis at 12 months of age or older. All of the group killed and examined at 18 months showed both conditions, as did all those in the 15, 16 and 17 month age groups on which autopsies were made because they were moribund or had died. Approximately 90 per cent of all age groups (11

to 18 months) had both amyloidosis and nephritis. Of the 12, 13 and 14 month groups, a few had nephritis without amyloid being apparent in the spleen or the duodenum. In a slightly greater number amyloid was found in the spleen and the duodenum without apparent degenerative changes in the kidney.

Comparatively few of the strain L mice had either amyloidosis or nephritis, and this number was decreased by excluding those which had apparent secondary amyloidosis, which occurred more often among the animals that had to be examined at the earlier ages. Only 3 of the 77 of the restricted group killed at 18 months showed the changes of the kidney, and in each of these 3 amyloid was also found. In 2 animals amyloid was found without the onset of renal changes. With advancing age the incidence of both conditions appeared to increase slightly, with possibly a preponderance of amyloidosis.

The first generation hybrids resulting from crossing strain A with strain L were comparable to the parent strain L. The combined data for the reciprocal groups show that only 1 animal of the 76 of the restricted lot killed and examined at 18 months had nephritis and only 3 had amyloidosis. Again as the age increased the incidence of each condition increased slightly, with the number of mice showing amyloidosis exceeding the number showing nephritis.

To test for a maternal influence reciprocal hybrids were produced. In the comparison of these two F_1 groups a difference was suggested, those with strain A mothers having a higher incidence of both amyloidosis and nephritis than those with strain L mothers. Statistically the differences of the data for the F_1 groups were borderline in significance: For amyloidosis $X^2=5.208$; $P=0.02$; for nephritis $X^2=3.18$; $P=0.07$. This difference, however, was not borne out in the reciprocal F_2 hybrids; hence the difference in the F_1 generation may not have been a real difference or, if real, not due to maternal factors that were passed on to the next generation.

In the F_2 generation evidence of segregation appeared. The LAF_2 and ALF_2 groups combined totaled 192, of which 159 were classified in the restricted group. Of these 159, 46, or 28.9 per cent, had nephritis. Considerably more, 76, or 47.8 per cent, had amyloidosis, and 43 of the 46 with nephritis showed amyloidosis. There was an increase in amyloidosis with increase in age, but in this group an increase in nephritis was not evident beyond 18 months.

In this generation there again appeared to be a difference in the two reciprocal groups, the incidence of both nephritis and amyloidosis being higher in the LAF_2 hybrids than in the ALF_2 . Furthermore, this difference was statistically significant; for nephritis $X^2=12.23$; $P<0.01$, and for amyloidosis $X^2=5.42$; $P<0.02$. The variation, however, was in the

direction opposite to that in the corresponding reciprocal F_1 groups, which makes the difference difficult to explain, if it is real.

In the generation that resulted from back-crossing the F_1 to the parent strain A, segregation of genetic factors was also apparent. In the restricted group there was a total of 97 of these back-cross animals of all age groups combined, and, of these, 51, or 52.6 per cent, had nephritis. Forty-eight of those with nephritis also showed amyloidosis, and an additional 14 showed amyloidosis without nephritis.

The results for the hybrids produced by back-crossing the F_1 to the parent strain L were comparable to those for the strain L and the F_1 generation in that the incidence of nephritis was low. Only 3 of the 69 animals of the restricted group showed nephritis, although considerably more, 18 of the 69, had amyloidosis.

The strain Y animals included in the data here were characteristic for the strain. All 4 had nephritis, although in 1 killed at 8 months amyloid was not found in the organs sectioned. Extensive amyloid was found in the 3 killed at 12 months.

Of the sample group of 90 F_1 hybrids produced by mating strain A females to strain Y males examined at 15 months of age, all showed extensive amyloidosis, with the degenerative changes in the kidneys classified as nephritis.

COMMENT

The data presented herein indicate that we are dealing with two closely associated degenerative conditions. Whether or not these conditions appear in an animal may be determined both by differences in non-genetic and by differences in genetic factors.

Strain differences are clearly indicated. One can expect practically all strain A mice that reach 15 months of age to show both conditions. While the number of animals of strain Y included in the work reported here is small, the experience we have had with the strain has shown in general that all of this strain would likewise show both conditions and at an age comparable to that noted for strain A. In contrast very few mice of strain L can be expected to present either condition. Such strain differences suggest the influence of genetic factors.

More conclusive evidence, however, of these genetic differences was found in the F_2 and back-cross generations that gave incidence rates indicating the results of the segregation of genetic factors. When the two high nephritis lines A and Y were mated, amyloidosis and nephritis developed in all the AYF_1 hybrids, but when a high nephritis line A was mated to a low nephritis line L, the ALF_1 and LAF_1 hybrids displayed the same low incidence that was observed in the low parent line, suggesting a recessive genetic factor or factors. By mating these ALF_1 and LAF_1 hybrids inter se, however, the incidence was raised as one would

expect, owing to the segregation of these genetic factors. Furthermore, when the F_1 animals were back-crossed to strain A, the incidence observed the A back-cross generation was higher than that in the F_1 , but the incidence in the L back-cross generation remained low, comparable to that in the L and the F_1 . Such a picture is conclusive evidence of segregating genetic factors.

The influence of nongenetic factors was also clearly indicated. Most prominent of these was chronic inflammation which resulted in secondary amyloidosis with tubular degeneration in the kidney, as mentioned earlier. Most of the animals in which this was a factor, however, could be identified by the presence of the chronic dermatitis or of other chronic inflammation together with the excess of amyloidosis of the spleen characteristic of the secondary type. Secondary amyloidosis was not apparent in the strain A groups, but none of this strain was kept beyond 18 months of age and the primary amyloidosis had developed in most of them at an even much earlier age. The secondary type was also not a factor in the few strain Y animals which were killed at a comparatively early age or in the AYF_1 hybrids killed at 15 months. It was, however, a factor in the strain L groups and the A by L hybrid groups; thus it necessitated the elimination of the data concerning these animals before further analysis of the data bearing on the causative factor of the primary condition was attempted.

The influence of additional and unknown nongenetic factors was evidenced in the cases of amyloidosis and nephritis remaining after the elimination of the identified cases of secondary amyloidosis in the strain L and LAF_1 groups. Individual variation in these groups could not be attributed to genetic variations, for these groups were genetically uniform and therefore individual differences would have to be attributed to variation in nongenetic factors.

The observation that amyloidosis occurred in connection with the nephritis in these cases substantiates Dunn's conclusion that the two conditions are related in that the tubular degeneration of the kidney results from obstruction due to the amyloid present in the intertubular connective tissue of the papilla. In most of the cases of nephritis amyloid was found in the spleen or the duodenum or both. In the few remaining cases it could have occurred in the kidney but was not apparent, owing to further degeneration of the papilla. More frequently amyloidosis was observed without renal changes, particularly in the older groups, indicating that the amyloid appeared earlier.

It is particularly important to emphasize the association of the two conditions in the hybrid groups where segregation of genetic factors is evident. This can be expected when two characters are linked or when both are due to the same genetic factor or factors. In this case, how-

ever, there is no evidence of linkage of genes for the two conditions but rather there is evidence that both result from the same gene.

The data indicate that but one pair of genetic factors is involved, with the gene for nephritis and amyloidosis recessive to the normal allele. The ratio of animals with nephritis to those without nephritis in the F_2 generation does not differ significantly from the 1 to 3 ratio expected with a single recessive factor. By comparing the observed with the expected incidence for the combined restricted groups one obtains an X^2 value of 0.787, with P between 30 and 50 per cent. Furthermore, the ratio in the strain A back-cross generation is not significantly different from the 1 to 1 ratio of a single recessive factor ($X^2=0.129$; P is between 50 and 70 per cent), and the incidence observed in the strain L back-cross is comparable to that in the parent strain L. Each group, however, has an excess of cases of amyloidosis, but this may be accounted for by non-genetic factors. The greater excess occurred in the older animals, an observation suggesting that in some of these cases the amyloidosis might have been secondary although the causative chronic disease was not identified.

While the data suggest a single recessive factor, a final conclusion cannot be drawn from these ratios alone. Ratios simulating those of single factor inheritance can occur in multiple factor inheritance where the presence or the absence of the character is determined by whether or not the combined effects of the genetic and nongenetic factors exceed a physiologic threshold. This condition was well demonstrated by Wright⁶ for polydactyly in the guinea pig and has been found also for the inheritance of pulmonary tumors in mice (Heston⁷). The possibility of this type of inheritance thus emphasizes the necessity of breeding tests of the back-cross or F_2 segregants to confirm single factor inheritance.

Since the renal changes evidently result from the deposition of amyloid, it is in the causation of the amyloidosis that one would look for the more direct effect of the genic action. In the light of the lack of knowledge of the metabolic disturbances resulting in the formation of amyloid, however, one hesitates to postulate a possible mechanism through which genetic variation may influence its occurrence. Aside from the secondary amyloidosis that accompanies some chronic diseases, experimental amyloidosis can be produced in mice by the injection of sodium caseinate as shown by Kuczynski⁸ and used by a number of later workers. In contrast it has been found⁹ that the primary amyloidosis

6. Wright, S.: *Genetics* 19:537, 1934.

7. Heston, W. E.: *J. Nat. Cancer Inst.* 3:69 and 79, 1942.

8. Kuczynski, M. H.: *Virchows Arch. f. path. Anat.* 239:185, 1922.

9. Heston, W. E.; Larsen, C. D., and Deringer, M. K.: *J. Nat. Cancer Inst.* 6:41, 1945.

characteristic of strain A is inhibited when mice of this strain are fed certain diets restricted in protein, particularly in protein yielding cystine. Amyloid is known to contain protein and probably arises through some disturbance of protein metabolism. Since much genetic action becomes manifest through enzymatic action, it seems logical that the amyloid described here could result from a recessive gene effecting an enzymatic deficiency in protein metabolism.

In discussing this renal condition in relation to those in man, Dunn suggested that the term "papilloncphritis" be applied, since it emphasizes the involvement of the papilla and does not suggest an analogy to any clearly differentiated type of nephritis in man. The relationship of the amyloidosis and the renal disorder, however, lays emphasis on the amyloidosis, since it is in the formation of the amyloid that one should seek the causative factors. Primary systemic amyloidosis occurs less frequently in man than secondary amyloidosis, but, as Iverson and Morrison¹⁰ have recently pointed out from their studies on cases of the primary condition, little has been indicated regarding the cause of the condition. The demonstration of the genetic factor causing amyloidosis in the mouse suggests that investigations directed toward discovering a genetic factor in primary systemic amyloidosis in man might be fruitful.

SUMMARY

These studies on the nephritis and amyloidosis that occur in mice of the high nephritis strains A and Y and the low nephritis strain L and in their hybrids confirm Dunn's suggestion that the two conditions are associated, the amyloidosis preceding the nephritis. Both genetic and non-genetic factors are demonstrated to be causative agents. Segregation of genetic factors was evident in the F_2 and strain A back-cross generations. The occurrence of some nephritis, although of low incidence, in the genetically homogeneous groups of strain L and the F_1 hybrids resulting from crossing strain L with strain A was evidence of the influence of non-genetic factors.

Outstanding among the nongenetic factors was a chronic dermatitis caused by the mite *Myobia musculi*, and that resulted in secondary amyloidosis. The secondary amyloidosis in turn resulted in nephritis.

Segregation ratios in the F_2 and back-cross generations suggested that the primary amyloidosis and the resulting nephritis were caused by a single recessive gene. Final conclusions, however, must await the results of breeding tests of back-cross or F_2 segregants.

These results suggest the desirability of studies in search of such a genetic factor as the causative agent of primary amyloidosis in man.

10. Iverson, L., and Morrison, A. B.: Arch. Path. 45:1, 1948.

VISCERAL LESIONS IN A CASE OF RHEUMATOID ARTHRITIS

PETER GRUENWALD, M.D.
NEW YORK

GRANULOMAS occurring in patients with rheumatoid arthritis have been examined by several investigators in order to demonstrate that the lesions are widely distributed throughout the body, and to compare these lesions with those of rheumatic fever. The subcutaneous nodules of both these diseases have been studied by Bennett, Zeller and Bauer,¹ who found many similarities, but also important differences, and concluded that "the nodules of rheumatoid arthritis and rheumatic fever differ as much from one another as do the granulomas of syphilis and tuberculosis." Granulomas have also been found in skeletal muscle² and along nerve trunks.³ Evidence of granulomatous disease in the hearts of patients with rheumatoid arthritis has been of great interest in view of the similarity of this and rheumatic heart disease. Baggenstoss and Rosenberg^{4a} frequently found lesions identical with the scars of rheumatic heart disease in cases of rheumatoid arthritis, without a history of rheumatic fever. In addition, the same authors^{4b} also found in 2 cases a different cardiac manifestation of rheumatoid arthritis, namely, large granulomas with necrotic centers, closely resembling the subcutaneous nodules occurring in the same disease. Similar granulomas were also present in one of these 2 cases in the pericardium and in the nearby portion of the wall of the aorta. Other visceral lesions in rheumatoid arthritis have, to the best of my knowledge, not been reported.

In view of the importance of these observations for the understanding of rheumatoid arthritis as a generalized disease, and for the elucidation of the relationship of this disease and rheumatic fever, the following findings of granulomas in the pleura and the peritoneum are reported.

From the Departments of Pathology, Long Island College of Medicine and Kings County Hospital, Brooklyn.

1. Bennett, G. A.; Zeller, J. W., and Bauer, W.: *Arch. Path.* **30**:70, 1940.
2. Steiner, G.; Freund, H. A.; Leichtentritt, B., and Maun, M. D.: *Am. J. Path.* **22**:103, 1946.
3. Freund, H. A.; Steiner, G.; Leichtentritt, B., and Price, A. E.: *Am. J. Path.* **18**:865, 1942.
4. Baggenstoss, A. H., and Rosenberg, E. F.: (a) *Arch. Path.* **35**:503, 1943; (b) **37**:54, 1944.

REPORT OF CASE

A 57 year old white man was admitted to Kings County Hospital with a six year history of rheumatoid arthritis. The disease began with a painful, hot swelling of the right shoulder. Subsequently, other joints were affected in a similar manner. The patient lost 18 Kg. (40 pounds) during these six years and was completely incapacitated during the sixth year. Ten days before admission the right shoulder again became swollen and tender. On admission the temperature was 98.6 F., the pulse rate was 84, and the blood pressure was 160 systolic and 98 diastolic. The right shoulder, elbow and knee were swollen and hot, but not red. The shoulder was tender. All other joints which could be examined, with the exception of the left shoulder, were swollen but not hot. The fingers were deformed, and the interossei muscles were atrophied. During the patient's stay in the hospital a decubitus ulcer developed over the sacrum. His temperature rose to 104.6 F., and he died thirty-three days after admission.

Laboratory observations, made shortly after admission, included: hemoglobin, 12 Gm. per hundred cubic centimeters; erythrocytes, 3,900,000, with white blood cells, 10,600, per cubic millimeter; Wassermann test, negative; blood urea, 46 mg., creatine, 1.7 mg., and uric acid, 4.2 mg., per hundred cubic centimeters. The urine was cloudy; tests for albumin and glucose showed none. Twenty-three days after admission the white blood cells numbered 20,000 per cubic millimeter, 85 per cent of which were polymorphonuclear leukocytes, 13 per cent lymphocytes and 2 per cent monocytes. Roentgenograms showed, in addition to the typical lesions of the joints of the extremities, marked hypertrophic arthritis with subluxation of the third and following cervical vertebrae, and destruction of carpal bones.

Autopsy.—The anatomic diagnosis was as follows: rheumatoid arthritis of many joints; granulomas of rheumatoid arthritis in the right atrium, the tricuspid valve, the pleura and the capsule of the spleen; partial fibrous obliteration of pericardial and pleural cavities; generalized arteriosclerosis; old obliteration of the left circumflex coronary artery; scarring following myocardial infarction of the left ventricle; arteriosclerotic aneurysm of the abdominal aorta; arteriosclerotic scarring of the kidneys; small pulmonary embolus; pulmonary emphysema and atelectasis; cortical adenomas of the kidneys; concretions in the bladder; diverticulum of the bladder; cystitis; fibroadenomas of the prostate; adenomas of the thyroid gland; decubitus ulcer over the sacrum. In the present discussion, only the findings of interest will be described.

Heart: The pericardial sac was partly obliterated by fibrous adhesions. The remaining cavity contained pale, turbid fluid. The free pericardial surfaces showed irregular hard thickenings and were covered with flakes of soft fibrin. The heart weighed 320 Gm. The chambers were opened in the usual manner. The right atrium showed on its inner surface in the area of the muscoli pectinati a large number of yellowish white firm thickenings, measuring approximately 2 mm. in diameter. One leaflet of the tricuspid valve showed a pale, firm thickening, which measured 1 cm. in diameter and 5 mm. in thickness. The remainder of the tricuspid valve, as well as the pulmonary and mitral valves, were not remarkable. The walls of the right ventricle measured 3 mm. in thickness and showed no significant changes. The leaflets of the aortic valve showed numerous arteriosclerotic plaques. The circumferences of the tricuspid, pulmonary, mitral and aortic valves measured 14, 6, 11 and 7.5 cm., respectively. In the wall of the left ventricle, near the apex there was an area in which the myocardium was replaced by fibrous tissue. This portion of the wall was markedly thinner than the rest and measured 6 mm. in thickness, whereas the remainder of the wall meas-

REPORT OF CASE

A 57 year old white man was admitted to Kings County Hospital with a six year history of rheumatoid arthritis. The disease began with a painful, hot swelling of the right shoulder. Subsequently, other joints were affected in a similar manner. The patient lost 18 Kg. (40 pounds) during these six years and was completely incapacitated during the sixth year. Ten days before admission the right shoulder again became swollen and tender. On admission the temperature was 98.6 F., the pulse rate was 84, and the blood pressure was 160 systolic and 98 diastolic. The right shoulder, elbow and knee were swollen and hot, but not red. The shoulder was tender. All other joints which could be examined, with the exception of the left shoulder, were swollen but not hot. The fingers were deformed, and the interossei muscles were atrophied. During the patient's stay in the hospital a decubitus ulcer developed over the sacrum. His temperature rose to 104.6 F., and he died thirty-three days after admission.

Laboratory observations, made shortly after admission, included: hemoglobin, 12 Gm. per hundred cubic centimeters; erythrocytes, 3,900,000, with white blood cells, 10,600, per cubic millimeter; Wassermann test, negative; blood urea, 46 mg., creatine, 1.7 mg., and uric acid, 4.2 mg., per hundred cubic centimeters. The urine was cloudy; tests for albumin and glucose showed none. Twenty-three days after admission the white blood cells numbered 20,000 per cubic millimeter, 85 per cent of which were polymorphonuclear leukocytes, 13 per cent lymphocytes and 2 per cent monocytes. Roentgenograms showed, in addition to the typical lesions of the joints of the extremities, marked hypertrophic arthritis with subluxation of the third and following cervical vertebrae, and destruction of carpal bones.

Autopsy.—The anatomic diagnosis was as follows: rheumatoid arthritis of many joints; granulomas of rheumatoid arthritis in the right atrium, the tricuspid valve, the pleura and the capsule of the spleen; partial fibrous obliteration of pericardial and pleural cavities; generalized arteriosclerosis; old obliteration of the left circumflex coronary artery; scarring following myocardial infarction of the left ventricle; arteriosclerotic aneurysm of the abdominal aorta; arteriosclerotic scarring of the kidneys; small pulmonary embolus; pulmonary emphysema and atelectasis; cortical adenomas of the kidneys; concretions in the bladder; diverticulum of the bladder; cystitis; fibroadenomas of the prostate; adenomas of the thyroid gland; decubitus ulcer over the sacrum. In the present discussion, only the findings of interest will be described.

Heart: The pericardial sac was partly obliterated by fibrous adhesions. The remaining cavity contained pale, turbid fluid. The free pericardial surfaces showed irregular hard thickenings and were covered with flakes of soft fibrin. The heart weighed 320 Gm. The chambers were opened in the usual manner. The right atrium showed on its inner surface in the area of the muscoli pectinati a large number of yellowish white firm thickenings, measuring approximately 2 mm. in diameter. One leaflet of the tricuspid valve showed a pale, firm thickening, which measured 1 cm. in diameter and 5 mm. in thickness. The remainder of the tricuspid valve, as well as the pulmonary and mitral valves, were not remarkable. The walls of the right ventricle measured 3 mm. in thickness and showed no significant changes. The leaflets of the aortic valve showed numerous arteriosclerotic plaques. The circumferences of the tricuspid, pulmonary, mitral and aortic valves measured 14, 6, 11 and 7.5 cm., respectively. In the wall of the left ventricle, near the apex there was an area in which the myocardium was replaced by fibrous tissue. This portion of the wall was markedly thinner than the rest and measured 6 mm. in thickness, whereas the remainder of the wall meas-

ured 14 mm. All coronary arteries showed marked arteriosclerosis, and their walls were rigid. The lumens were wide and gaping; only the left circumflex artery was narrow and markedly calcified. It was completely obliterated about 1 cm. from its origin from the main artery.

Aorta: The entire aorta showed arteriosclerosis, which was especially severe in the descending portion. There were numerous large, firm plaques, some of them calcified or ulcerated. Below the level of the origin of the inferior mesenteric artery there was a spindle-shaped dilatation in which the diameter was twice that of the rest of the vessel. In this area there were numerous ulcerated plaques partly covered by thrombotic material which formed a layer 0.5 cm. in thickness.

Lungs: The left pleural cavity was obliterated by fibrous adhesions with the exception of a small pocket which contained fibrin and turbid fluid. The upper portion of the right pleural cavity was similarly obliterated, whereas the lower portion contained a yellowish, turbid fluid and large amounts of fibrin. The surface of the lung in this area showed irregular hard thickenings and was covered with masses of soft fibrin. The left lung weighed 470 Gm.; the right lung, 430 Gm. The anterior portions of both lungs were pale and emphysematous, whereas the posterior portions were red and firm. A large portion of the lower lobe of the right lung was firmly consolidated and reduced in size. Its cut surface was pale red. Several small calcified nodules were scattered irregularly throughout both lungs. The mucosa of all bronchi was red and swollen. A small embolus was found in an artery of the lower lobe of the left lung. The hilar lymph nodes were anthracotic.

Spleen: The spleen adhered to neighboring organs at several points. The greater part of its surface was covered with white fibrous tissue. The weight of the organ was 330 Gm.; the consistency was firm. On section the tissue was dark purple and showed distinct trabeculae and follicles.

Joints: Both sternoclavicular joints had enlarged cavities, which contained soft, pale yellow, fatlike material. The articular surfaces were rough. Of the larger joints, only the right shoulder was examined. The capsule of the joint was irregularly thickened. The articular surfaces showed many defects in their cartilaginous linings. A large cavity next to the joint was filled with a brown, viscid, somewhat turbid fluid. Several cervical vertebrae were compressed in a cranio-caudal direction.

Microscopic Examination.—Tissues were fixed in Zenker's fluid and embedded in paraffin. Sections were stained with hematoxylin and eosin and, whenever desired, with any of the following methods: Masson's trichrome stain, Gömöri's silver impregnation of reticulum, Gram stain, and stain for acid-fast bacteria. Stains for bacteria revealed no bacteria and will therefore not be described.

The heart showed in the right atrium and in the tricuspid valve large granulomas, which were seen as nodules on gross examination. The muscular wall of the right atrium was completely replaced in some areas by this granulation tissue (fig. 1). A typical unit of the abnormal tissues (fig. 5) consisted of a granuloma with an area of necrosis in the center. The necrotic tissue stained red with hematoxylin and eosin and showed no cell bodies or nuclei. Only fine blue-staining particles were present, which were probably remnants of fragmented nuclei. In some of the necrotic areas these bluish-stained particles were limited to the border. The necrotic tissue was surrounded by the granulation tissue, in which two zones were distinguished, which were identical with those described by Baggenstoss and Rosenberg.^{4b} The inner zone consisted of large oblong cells in a radial arrange-

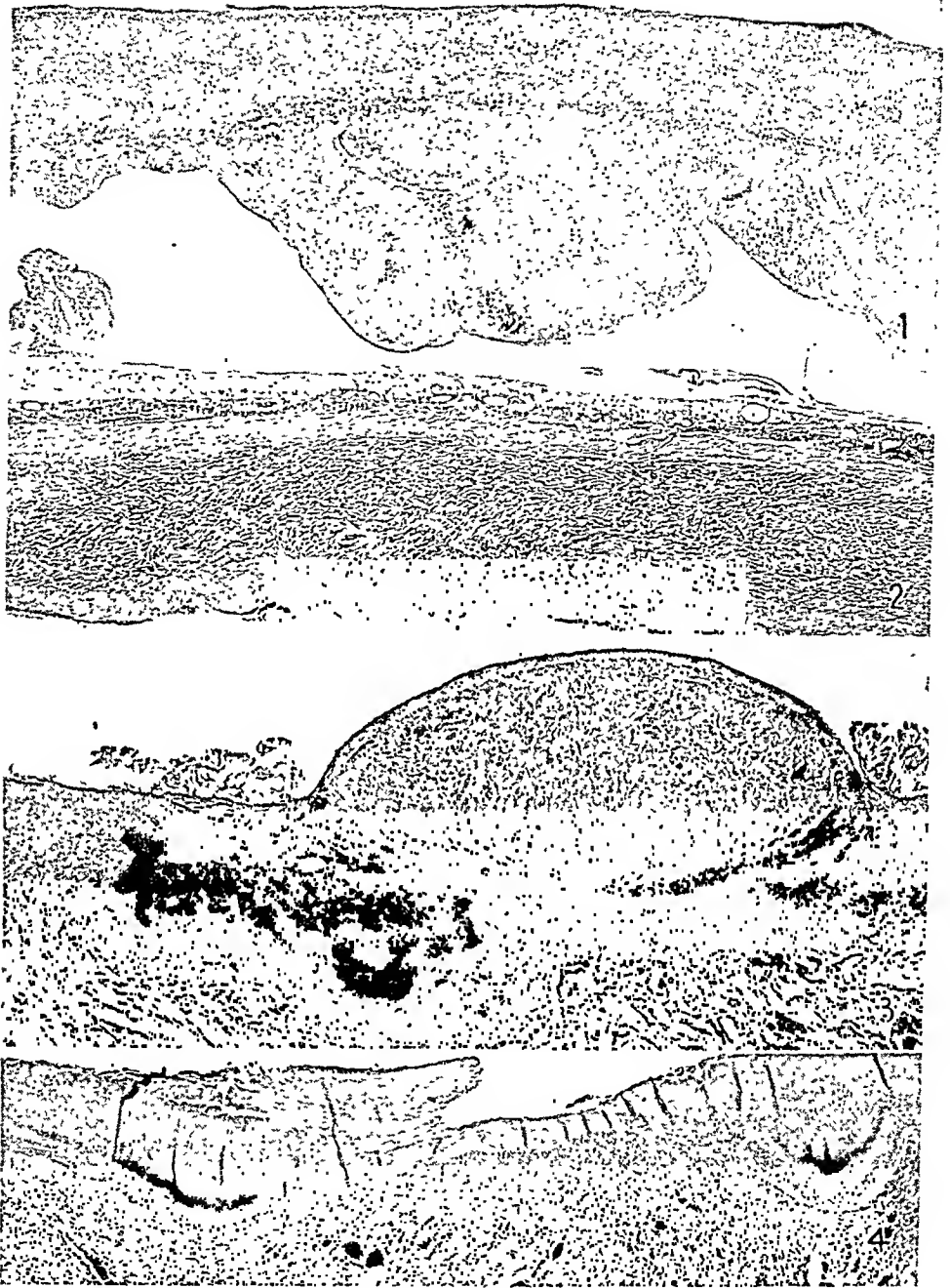


Fig. 1.—Right atrium showing a mass of granulation tissue protruding into the lumen. Note the several areas of necrosis in the granuloma. Hematoxylin and eosin stain; $\times 18$.

Fig. 2.—Parietal pericardium with a layer of dense fibrous tissue. The lower surface in the figure faces the pericardial cavity. Masson stain; $\times 18$.

Fig. 3.—Lung with several large granulomas in the pleura. The node protruding on the surface is not surrounded by granulation tissue. The other node, located deeper and to the left of it, contains dark-staining, more recent areas of necrosis surrounded by granulation tissue. Hematoxylin and eosin stain; $\times 18$.

Fig. 4.—Surface of the spleen with a layer of granulation tissue. The necrotic portion of the latter is on the surface; the granulation tissue, between it and the pulp. Hematoxylin and eosin stain; $\times 18$.

ment. These cells had large, pale-stained nuclei, which might be rod shaped or somewhat lobulated. Interspersed between the cells were polymorphonuclear leukocytes and lymphocytes. Some of the large cells had apparently fused with one another and given rise to giant cells. The arrangement of the nuclei of some of these giant cells was irregular; in others the nuclei were arranged in the form of a sphere as is characteristic of the Langhans giant cell. The outer layer of granulation tissue had an uncharacteristic appearance and consisted of fibroblasts with large nuclei resembling those of the cells in the inner zone, lymphocytes and small numbers of polymorphonuclear leukocytes. This granulation tissue blended without a definite border into that of nearby nodules or into the surrounding connective tissue. The development of the granulomas appeared to be in different stages at various parts of the circumference. In some areas, the border of the necrotic zone showed many nuclear fragments, and the adjacent palisading cells were well developed. In other areas, these cells could hardly be distinguished, and the necrotic tissue was almost in contact with the outer layer of granulation tissue. The large node in the tricuspid valve showed particularly little growth activity. In some portions of its circumference the granulation tissue was almost absent, and in others it was reduced to a row of large foam cells. Some of these foam cells were multinucleated, which suggested that they had arisen from the large palisading cells of the granulation tissue. The nuclei of the small foam cells also resembled those of the cells in the granulation tissue. There was but minimal lymphocytic and plasma cell infiltration. Mallory's stain for collagenous fibers and Gömöri's silver impregnation for lattice fibers revealed large numbers of both types of fibers in all layers of the granulomas. The arrangement of these fibers was more clearly seen in granulomas of the spleen and will be described there.

Several sections of the visceral and the parietal pericardium showed diffuse inflammation. In some areas there was a granulation tissue of uncharacteristic appearance which contained foam cells resembling those seen in the tricuspid valve. In other areas there was a thick layer of dense fibrous tissue which showed on the surface alternating elevations and depressions (fig. 2). An arrangement of the coarse fiber bundles in whorls was apparent at many points, resembling that in the center of the large granulomas. In one of these areas in which the pericardial lumen was obliterated except for a few small clefts lined by cuboidal epithelium, the fibrous tissue was covered by a thin layer of granulation tissue. The cells closely resembled those in the granulomatous nodules, and a few large multinucleated cells were also present (fig. 6). The arrangement of the connective tissue fibers and the presence of this granulation tissue suggested that in these portions of the pericardium a process had occurred which was similar to that in the nodular granulomas except that it was spread out along a serous membrane rather than concentrated in the form of nodules.

The aorta showed severe arteriosclerotic changes, which are not to be described here. Only one observation must be mentioned. The adventitia showed an area of thickening and fibrosis which somewhat resembled a granuloma. The center of this area contained few cells, and in some portions it was entirely acellular. Surrounding this center was an uncharacteristic granulation tissue, which consisted mostly of small fibroblasts and a few round cells. This nodule was distinctly different from the characteristic granuloma of rheumatoid arthritis, and their relation could not be ascertained. Several other sections of the aorta showed no similar nodules.

The lungs were the seat of numerous areas of acute and chronic inflammation which is of no concern here. The pleura was thickened by the presence of granulation tissue and fibrosis, and in many areas these changes were of the usual

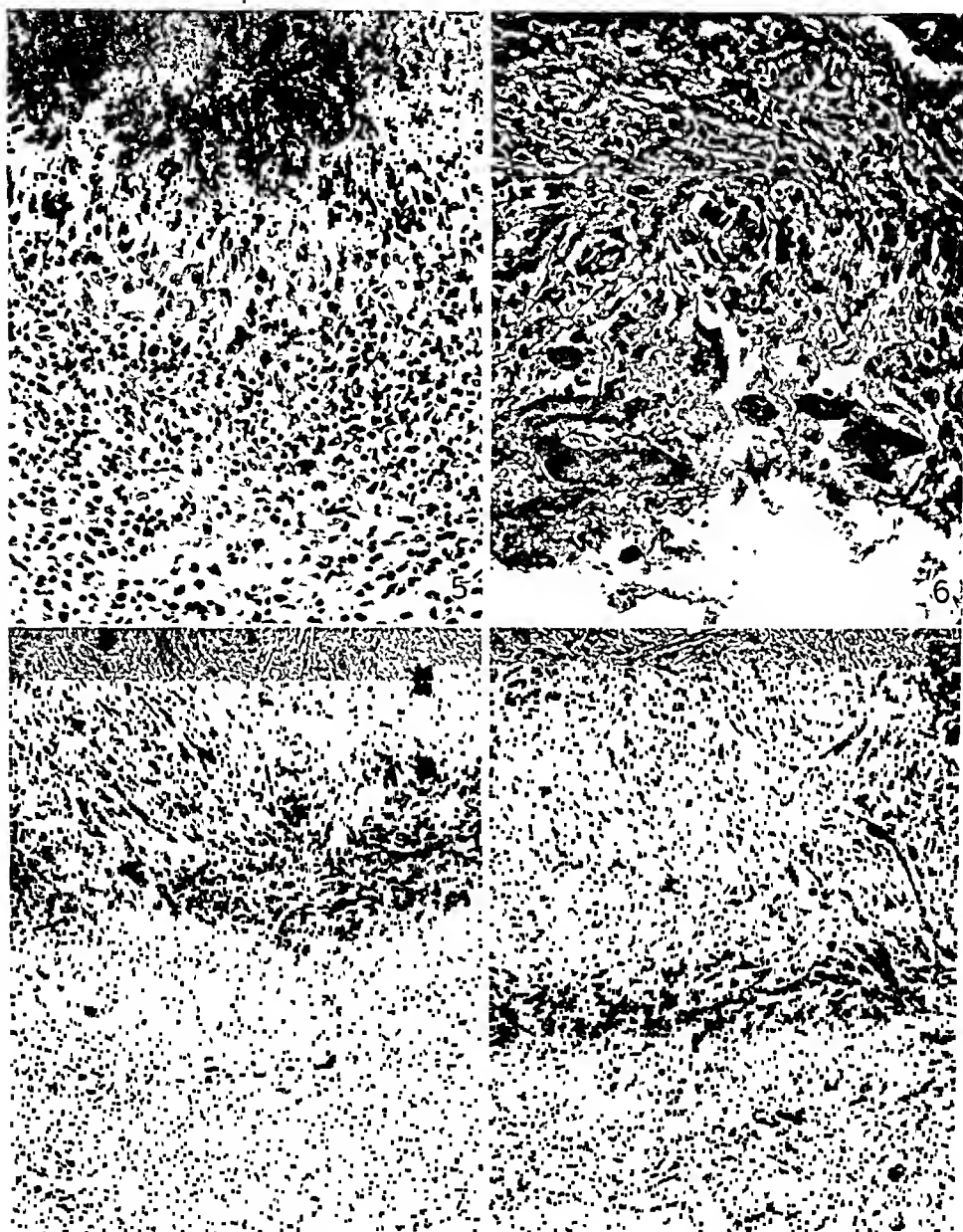


Fig. 5.—Right atrium, showing with higher magnification one of the areas of necrosis seen in figure 1. The nearby granulation tissue is seen in the lower portion of the figure. Hematoxylin and eosin stain; $\times 250$.

Fig. 6.—Pericardium showing the border of the fibrous layer (see figure 2) lined by granulation tissue resembling that of the nodular granulomas. Hematoxylin and eosin stain; $\times 280$.

Fig. 7.—Portion of the layer of granulation tissue on the surface of the spleen (see figure 4). The upper part of the figure shows the area of necrosis; the lower part, the granulation tissue proper. Hematoxylin and eosin stain; $\times 140$. (The dark spots in the upper part are artefacts.)

Fig. 8.—Section of the same block as the section in figure 7, with Masson stain; $\times 140$.

nonspecific appearance. In other parts there were large granulomatous nodes which closely resembled those found in the right atrium (fig. 3). They also showed single or confluent areas of necrosis surrounded by a granulation tissue with fairly numerous giant cells. It was common to find granulation tissue lining only that side of the necrotic areas which faced the lung tissue, whereas the other side was adjacent to fibrous tissue. At one point the layers of the granulation tissue were arranged parallel to the surface of the organ rather than in the form of a node. The necrotic portion was nearest the surface, and the granulation tissue was found between it and the lung tissue. This may well have represented an earlier stage of the fibrosis described in the pericardium. The necrotic tissue in a large group of confluent nodes contained a large amount of anthracotic pigment. Another node was distinguished by the complete absence of granulation tissue. The necrotic area was surrounded by fibrous tissue everywhere (fig. 3). This resembled somewhat the node found in the tricuspid valve.

The spleen showed extensive thickening of its capsule due to the presence of granulation tissue both in the form of nodules and of layers parallel to the surface (fig. 4). In both instances there was the same sequence of necrotic tissue, palisading large fibroblasts and uncharacteristic granulation tissue (fig. 7). The arrangement of the connective tissue fibers in the necrotic areas was similar to that seen in the heart and the pleura, but its features were best recognized in sections of the spleen. As has been mentioned, stains for collagenous and lattice fibers revealed large numbers of both (fig. 8). They formed bundles, which were arranged irregularly or in whorls. The interior of the spleen showed nothing of interest.

The head of the humerus showed an irregular articular surface, which was covered by cartilage only in some scattered areas. At other points the marrow spaces were separated from the joint cavity only by thin trabeculae of bone and a narrow layer of connective tissue. The synovial membrane was thickened by the presence of a nonspecific granulation tissue which contained large numbers of foam cells. No typical granulomas were present, but in a few areas the foam cells formed small nodules which also contained small round cells and moderate numbers of fibroblasts. In the center of one of these nodules there were a number of foam cells which were large and multinucleated. Within the bone of the humerus there were several nodules consisting of large fibroblasts and foam cells, and between them spindle-shaped cavities which had the shape of cholesterol crystals. In several of these nodes a central area was occupied entirely by debris with spindle-shaped cavities.

COMMENT

There is no direct proof that in the present case the visceral lesions are those of rheumatoid arthritis. However, this relationship is suggested by the fact that the patient had rheumatoid arthritis, as well as by the close resemblance of the granulomas in the viscera to those commonly seen in the subcutaneous tissue in rheumatoid arthritis. Furthermore, the granulomas observed in the heart conformed with those previously found in 2 cases of this disease by Baggenstoss and Rosenberg. Staining methods revealed no bacteria in tissue sections. For these reasons it will be assumed in the following discussion that the lesions under consideration are part of the generalized disease associated with rheumatoid arthritis.

It has been the purpose of this report to demonstrate the hitherto unknown fact that granulomas of rheumatoid arthritis may be located in the pleura and the peritoneum. Since similar granulomas had previously been found in the pericardium,^{4b} and since there were also indications that a similar process may have gone on in the pericardium in the present case, the observation that such granulomas occur in all serous membranes is now on record. There were shown in the present case, also, granulomas in the heart which conformed with those described by Baggenstoss and Rosenberg^{4b} except that they were located in the right side of the heart.

It is noteworthy that the granulomas in question, which largely conformed in their structure to those described in the subcutaneous tissue and in the heart, are often found in approximately the same condition. This suggests, as Bennet, Zeller and Bauer¹ have stated, that the process of development of these nodules is a slow one. Only one of the nodules in the tricuspid valve and one in the pleura of the present case differed from the others by showing no border of palisading cells and granulation tissue. In the typical granulomas, necrosis in a large central area affects a preexisting granulation tissue rather than a previously normal tissue, as can easily be demonstrated by stains for connective tissue. The fibers which are demonstrated by these methods are arranged in whorls and show no indication of the structure of the previous normal tissue in that area. It is peculiar that these staining qualities persist in the necrotic centers of all nodules even though some of these are presumably of long standing.

There are indications that a type of growth similar to that of the nodules may also occur in a continuous layer spread out over large areas of serous surfaces. In that event the various layers of the granuloma are arranged roughly parallel to the surface of the organ rather than concentrically. It is probable that in a late stage of development these formations give rise to a thick layer of firm fibrous tissue in which the original whorl-like arrangement of the fibers can still be seen.

A few previous reports may be mentioned which possibly deal with changes similar to those in the present case. Bennett, Zeller and Bauer¹ referred in passing to a case in which lesions resembling the subcutaneous nodules of rheumatic fever (as opposed to rheumatoid arthritis) were found in the pericardium and in the pleura of a patient with rheumatoid arthritis. Fingerman and Andrus⁵ studied the visceral lesions in 61 cases of rheumatoid arthritis and found hyaline perisplenitis in 8 cases and marked fibrous pleural adhesions in 23. The distribution of these lesions corresponds well with that of the granulomas in the present case, and this is significant since dense fibrosis of the pericardium in the present

5. Fingerman, D. L., and Andrus, F. C.: *Ann. Rheumat. Dis.* 3:168, 1943.

case showed indications of being derived from the characteristic granulation tissue of rheumatoid arthritis.

SUMMARY

Autopsy observations in a case of rheumatoid arthritis of six years' duration are described with particular reference to visceral lesions. Granulomas identical with those known to occur in the subcutaneous tissue and in the left side of the heart were found in the right side of the heart, the pleura, and the capsule of the spleen. This indicates, in conjunction with previous reports, that all serous membranes are subject to lesions of rheumatoid arthritis, as well as both sides of the heart and other previously known locations.

Case Reports

EWING'S ENDOTHELIAL MYELOMA OF ADOLESCENTS

Report of Two Fatal Cases

HORACE K. GIFFEN, M.D.

Pathologist, Youngstown Hospital Association Laboratories

YOUNGSTOWN, OHIO

ABOUT a quarter of a century ago Ewing¹ described a tumor of bone which he called "diffuse endothelioma of bone." The condition is found mainly in children, with the first evidence of the disease occurring usually as fever, pain, tenderness and leukocytosis. It is often confused with osteomyelitis. The primary site is frequently in the diaphysis of one of the long bones. There are marked osteolysis spreading along the shaft of the bone, expansion and perforation of the cortex, and a process extending along the periosteum into the soft tissues. The neoplasm probably spreads by the blood stream but may involve regional lymph nodes. Other bones become involved either through metastasis or, as some believe, through multicentric development of the disease.² Eventually metastases are found, usually in the lungs and occasionally in many organs. The tumor is temporarily radiosensitive, but radiation offers no cure for Ewing's tumor. The present view favors considering the disease as cancer of the reticuloendothelial system, a view which is not far removed from the original concept held by Ewing. Specimens of the tumor vary somewhat in cytologic detail but present a fairly distinct clinical entity.

REPORT OF CASES

CASE 1 (reported with the permission of J. D. Brown, M. D., and R. R. Morrall, M. D.).—R. J., a 13 year old white boy, first noticed pain in his right thigh in the early days of August 1945. The pain was severe at night and kept him awake. August 9 he was playing baseball when, without any direct trauma, his leg gave way and he fell. On being admitted to the Youngstown Hospital he was found roentgenologically to have extensive medullary and focal cortical absorption of the right femur, with a pathologic fracture near the junction of the middle and upper thirds of the bone. There were also periosteal elevation and proliferation along the shaft of the bone for several inches proximal to the fracture. The

1. Ewing, J.: *Neoplastic Diseases*, ed. 4, Philadelphia, W. B. Saunders Company, 1940.

2. Harvey, W. F.; Dawson, E. K., and Innes, J. R. M.: *Debatable Tumours in Human and Animal Pathology*, London, Oliver & Boyd, Ltd., 1940.

roentgenologic findings were interpreted as characteristic of Ewing's tumor. No other bones were found involved at that time. A few days later a biopsy confirmed the diagnosis of Ewing's endothelial myeloma. During the early days in the hospital there was occasional mild fever, the temperature rising to a maximum of 101 F. The pulse rate corresponded, with mild elevation.

The erythrocyte count was 3,950,000; the hemoglobin content was 11.5 Gm. The leukocyte count was 5,800, with a differential count showing 70 per cent polymorphonuclears and a slight shift to the left. The serum phosphorus was 4.9 mg., and the blood calcium 10.397 mg., per hundred cubic centimeters. The Kahn and Kline tests revealed no syphilis. The urine had a specific gravity of 1.027; it contained no albumin, no sugar and no Bence Jones Protein.

Roentgen therapy was given to the maximum tolerance—a total of 8,496 roentgens. By the third month there was partial healing of the fractured bone. By the fifth month pain had developed in the frontal region of the head and in the left mandibular region. He was transferred to the Memorial Hospital for the Treatment of Cancer and Allied Diseases, New York, for several weeks. There he received seventeen injections of Coley's toxins (an unfiltered mixture of erysipelas and *Bacillus prodigiosus* cultures) and additional roentgen therapy. He was readmitted to the Youngstown Hospital in the eighth month after symptoms had begun. Pain was generalized by that time in both extremities and in his head. The blood pressure became elevated to 160 systolic and 100 diastolic. There was little or no fever. Morphine had to be given daily for severe pain. By the eleventh month he was completely blind in the left eye, and vision was reduced in the right eye. He became emaciated and weak and died about fourteen months after the appearance of the initial symptoms.

Autopsy.—The body was that of an emaciated 14 year old boy. There was a tumor involvement of the upper part of the right femur, the skull, the right clavicle, both tibias, the left fibula and the left second rib. Also involved were soft tissues as follows: both lungs, the small intestine, the pancreas, the mesenteric lymph nodes, both kidneys, one adrenal gland, the dura mater, the leptomeninges over the cerebrum and, slightly, the outer part of the cerebrum. Both lungs showed bronchopneumonia. Histologically, the tumor was fairly uniform in the various sites where it was found. The cells were found in solid sheets or masses; they were fairly large cells, often rounded, with large hyperchromatic nuclei, which were frequently vesicular and occasionally showed a nucleolus. The nuclear border was usually well defined, and the cytoplasm of the cell was scanty. In many nuclei the chromatin was clumped. There was little intercellular substance in most masses of the tumor. Some of the lobules of the tumor were separated by a small amount of connective tissue. Silver stains showed no reticulum fibrils in close relationship to the tumor cells. In some masses of the tumor there was striking rosette arrangement of the tumor cells, best seen in the masses found in the lungs. The rosette was composed of a central pale fibrillar mass surrounded by roughly radiating cells. No blood vessels could be detected in these central zones in most places. Vascularization was abundant in most of the neoplastic masses, with many areas showing collections of blood or blood sinusoids lined by tumor cells. Occasional veins showed tumor thrombi. No tumor was evident in the spleen, but in the spleen there was extensive evidence of destruction of blood, with many histiocytes loaded with iron-bearing blood pigment. The tumor which remained in the right femur was altered considerably by necrosis, apparently caused by the heavy roentgen irradiation.

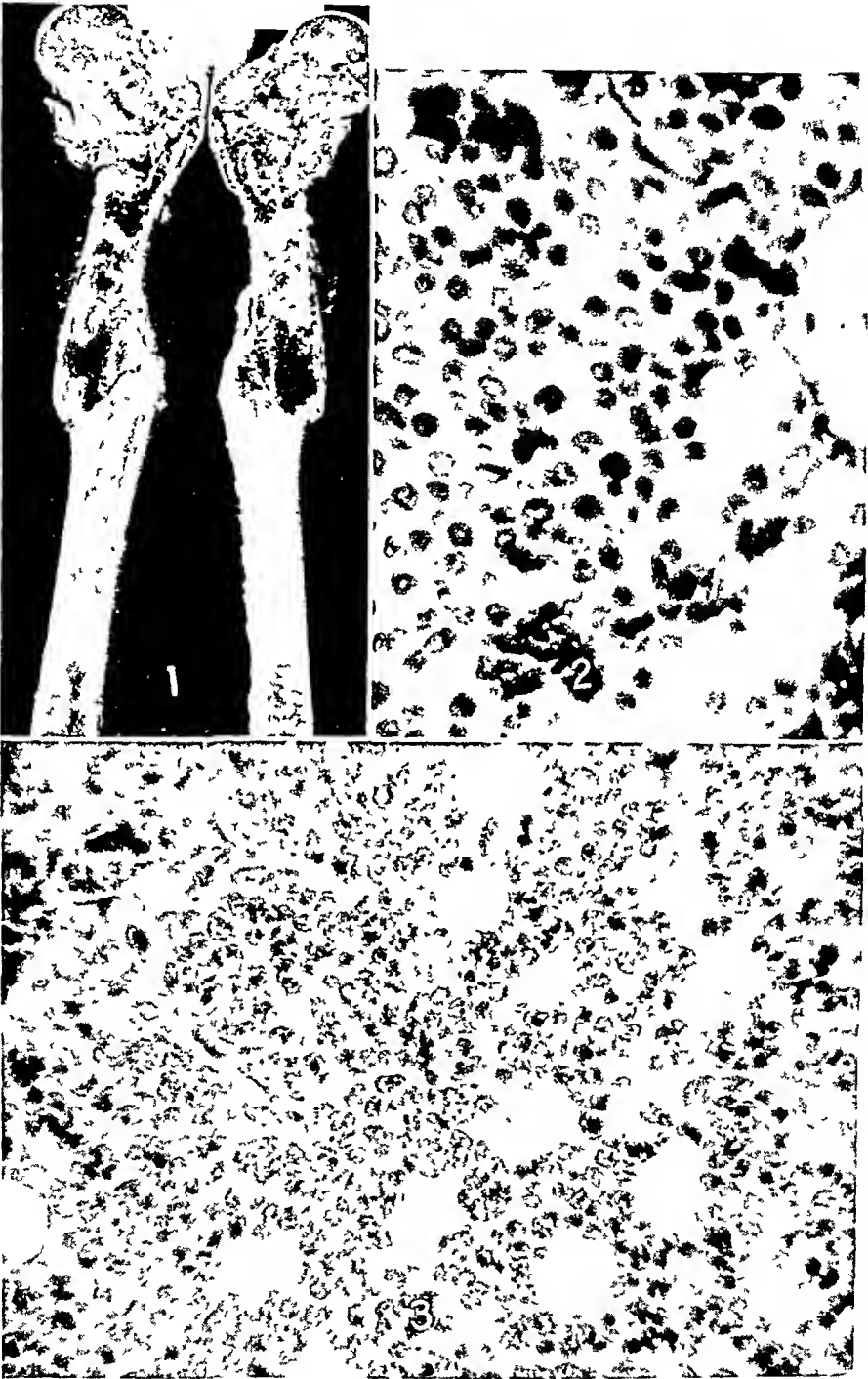


Fig 1 (case 1) —Femur as seen at death. It shows extensive neoplastic involvement above and below the fracture, which is partially healed

Fig 2 (case 1) —Metastatic tumor of the lung showing abundant rosette formation and some condensation of tumor cells about blood vessels

Fig 3 (case 2).—Pulmonary tumor nodule stained with silver. There is no reticulum in most of it and none intimately related to the tumor cells. Cytoplasm is scanty.

CASE 2 (reported with the permission of R. R. Morrall, M.D.).—A 16 year old white girl first felt discomfort in her left shoulder while playing baseball about May 1, 1946. She did not remember that her shoulder was actually injured, but pain began at that time. She was treated by a chiropractor for about two months. The soreness in her left shoulder was poorly localized. When she was admitted to the Youngstown Hospital, July 29, there was a swelling which seemed to elevate the left scapula. Roentgenologic examination revealed a zone of irregular destruction of bone in the body of the left scapula with some elevation of the regional periosteum. A biopsy showed Ewing's tumor with invasion of the surrounding muscles.

The erythrocyte count was 4,890,000; the hemoglobin content was 14.5 Gm. The leukocyte count was 4,450, with a normal differential count. The serum phosphate was 4 mg. per hundred cubic centimeters, and the serum phosphatase, 4.9 units—both within normal limits. The Kahn and Kline tests of the blood revealed no syphilis. The urine contained no albumin, sugar or cells, and no Bence Jones protein was evident.

On examination no other bone could be detected in which there was any abnormality. After a biopsy, heavy roentgen radiation was applied locally over the left scapular region. The patient seemed to improve clinically during the first few weeks. However, about three months after the initial symptoms had been noted, she began to experience discomfort around her right knee. Soon discomfort developed in the lower lumbar region of the back. By the fourth month she had a low grade fever, but the blood count and the urine remained normal. A roentgenogram revealed focal bony destruction in the third lumbar vertebra. She lost weight rapidly, and a soft tissue mass began to develop over the left midparietal area of the skull. Soon roentgenograms showed osseous destruction in this region and also in the distal end of the right femur. Generalized pain became marked. Both eyes became swollen, and blindness developed in the left eye. She died about six months after she had noted the first symptoms.

Autopsy.—The body was that of a well developed but emaciated 16 year old white girl, with a pale skin. There were petechiae over the thorax and the abdomen. A tumor involved the left scapula, the skull on both sides, several ribs, thoracic and lumbar vertebrae and the right femur. There were metastases of the tumor in both the lungs and the pleurae, in the pancreas, in the pituitary gland and in the dura mater and the leptomeninges, with slight invasion of the upper part of the cerebrum. Histologically, the tumor was found in solid sheets, rounded, compact nodules, or strands. The cells were moderately large and rounded or pleomorphic; they had scanty cytoplasm and round or oval nuclei, which were often vesicular. The chromatin was irregular in distribution, and the nucleoli were inconspicuous. Tumor thrombi were scattered in the lungs, and solid masses of neoplasm filled many of the alveoli. There was little tendency to form rosettes in this tumor. In most places, intercellular substance was lacking, and reticular stains revealed no intimate relationship between the interlobular connective tissue reticulum and the tumor cells themselves. Scattered hyperchromatic nuclei were present, and a few atypical mitoses were seen in some nodules. The masses of tumor cells showed marked vascularization, with many blood sinuses lined by tumor cells.

COMMENT

These 2 cases of Ewing's tumor are fairly typical. Both patients were adolescents. Both related their first symptoms to playing baseball, but

neither showed clear evidence of direct trauma. In the boy the original pain, swelling and bony lesion were in the femur, while in the girl the neoplasm seemed to originate in the left scapula. In both cases there was an interval of several weeks or months before lesions were evident in other bones or tissues. Both patients had been in apparently good health prior to the onset of the disease.

Ewing believed that the tumor originated from the endothelium of the medullary capillaries of the bones. Present writers definitely relate it to the reticuloendothelial system, and therefore their observations are not far removed from Ewing's original concept. Hadfield³ classed Ewing's tumor under reticulosarcoma and considered it along with some other tumors as undifferentiated mesenchymal tissue found in syncytial arrangement or as tumors derived from the primitive mesenchyme which show varying degrees of differentiation. Some of the tumors show reticulin fibrils; some seem to show immature lymphocytes or lymphoblastic tissue and some show sheets of cells with little evidence of differentiation. Ewing's tumor is osteolytic but does not produce bone in itself. It does penetrate the cortex and tends to expand it. It may cause considerable proliferation and slight new bone formation in the subperiosteal region of the involved primary site. In some instances it is composed of larger cells than the ones illustrated in our cases. Hadfield described the characteristic cells as having copious, faintly staining cytoplasm and a vesicular nucleus such as is seen in the reticulum cells. Usually the fairly uniform cells are closely packed, with little intercellular substance. Apparently Oberling⁴ was the first to relate the tumor to the reticuloendothelial system. He defined four different types showing various degrees of differentiation.

Willis⁵ suggested that at least in some of the clinical cases Ewing's tumor may be secondary to an inconspicuous primary focus of neoplastic growth in one of the internal organs. It may originate in the adrenal gland, in nerve tissues or in the lungs. It is true that the tendency to form rosettes in many of the cases suggests a neurogenic type of tumor. In our cases there was found no evidence to substantiate the belief that the origin of the tumor was at any site other than that described. The fact that swelling and pain occurred at the site of the original lesion seems to indicate that this was the true site of origin. One of our patients did have a tumor metastasis in the cortex of one of the adrenal glands, but

3. Hadfield, G., and Garrod, L. P.: *Recent Advances in Pathology*, Philadelphia, The Blakiston Company, 1947.

4. Oberling, C.: *Bull. Assoc. franç. p. l'étude du cancer* 17:259, 1928.

5. Willis, R. A.: *The Spread of Tumours in the Human Body*, London, J. & A. Churchill, Ltd., 1934.

no evidence could be found to indicate that the tumor was in either the medulla or any other part of the nervous system.

Phemister⁶ attempted to separate Ewing's sarcoma from reticulum cell sarcoma, angiosarcoma and lymphosarcoma occurring in the bones. The first two and the last, however, all are seen most frequently in children and seem poorly defined from his description and illustrations. It seems reasonable to class them together as varying only in the degree to which they have differentiated from the multipotential mesenchyme from which they apparently originate.

SUMMARY

Two fairly typical cases of Ewing's tumor are presented, with the clinical, histologic and radiologic characteristics. The tumor is accepted as a cancer of that part of the reticuloendothelial system which is located in the medullary regions of bones, especially in those of the diaphyses of long bones.

6. Phemister, D. B.: J.A.M.A. 136:550, 1948.

DYSCHONDROPLASIA WITH HEMANGIOMATOSIS (MAFFUCCI'S SYNDROME)
AND TERATOID TUMOR OF THE OVARY

J. F. KUZMA, M.D.

AND

J. M. KING, M.D.
MILWAUKEE

THE COMBINATION of dyschondroplasia and hemangiomatosis is exceedingly rare. Carleton and associates¹ were able to collect 18 cases from the European literature, and to this group they added 2 cases of their own. Krause² recorded the first case to be described in the American literature, in 1944. Carleton and associates¹ suggested that the name of Kast³ be associated with the syndrome. However, in 1942, after a further study of the literature, they discovered that the first recognizable case of dyschondroplasia associated with hemangiomatosis was described in 1881 by Maffucci, and his name was then given to the syndrome.

Dyschondroplasia, or Ollier's disease, is a condition affecting the growing ends of the bones. Normal ossification of cartilage does not take place, and as the bone increases in length, the areas of cartilage which fail to ossify persist in the metaphysis. There is dwarfing of the limbs, with irregular bending, and there are multiple nodular tumors. The condition is commonly unilateral or markedly asymmetric. Maffucci's syndrome differs from Ollier's disease in that there is present the additional element of hemangiomatosis.

Dyschondroplasia can be regarded as a nonhereditary mesodermal dysplasia which becomes clinically evident before puberty. The reported cases concerned 15 males and 6 females. The patients' ages at the dates of recording ranged from 8 to 58 years—with an average of 33 years. According to the report of Carleton and associates,¹ the usual history is as follows: The child is apparently normal at birth, but sometime in the years before puberty, from the first to the twelfth year, a hard nodule, 1 to 2 cm. in diameter, appears on a finger or a toe to be followed soon afterward by other nodules on the feet and the hands and on the legs and arms. The vertebrae, the ribs, the scapulas and the pelvis may be the

From the Departments of Pathology and Surgery, Milwaukee County Hospital and Marquette University School of Medicine.

1. Carleton, A.; Elkington, J. St. C.; Greenfield, J. G., and Robb-Smith, A. H. T.: *Quart. J. Med.* 11:203, 1942.

2. Krause, G. R.: *Am. J. Roentgenol.* 52:620, 1944.

3. Kast and von Recklinghausen: *Virchows Arch. f. path. Anat.* 118:1, 1889.

sites of tumors, but the skull, the insteps and the wrists are rarely involved. These hard nodules are identified as enchondroma. At the time at which they appear, soft, bluish tumors (hemangioma) develop on the affected limbs. Large, dilated veins may be found associated with the soft tumors.

The distribution of the tumors (enchondroma and hemangioma) may be extremely asymmetric, but it is rarely absolutely unilateral. Skeletal development may be retarded on one side; one or more long bones may have short shafts with irregularly expanded ends and cartilaginous tumors at the epiphyseal lines. Trivial injuries may cause fractures of the long bones, with delayed callus formation or nonunion.

The deformities may increase throughout the period of development, and in severe cases the hands and the feet may become transformed into such huge enchondromatous masses as to be almost unrecognizable. The patient presents an extremely grotesque appearance, and the affected limb or limbs may be so large that all function is lost, and amputation becomes imperative for comfort.

In the early twenties the condition becomes stationary, and if there has been only a moderate degree of deformity, which may necessitate only loss of a finger or a toe, the affected person can be considered fortunate. In several instances, however, injuries occurring later in life have caused new nodules to appear. There is no pain associated with either the hard or the soft tumors.

Amputations were carried out in almost one half of the reported cases. Chondrosarcoma was noted in 4 instances. Other reported changes included abnormal sweating, vitiligo and cerebral glioma.

REPORT OF A CASE

S. S. was a 19 year old dwarfed white girl of Polish descent, not married. She presented herself in June 1943 with a gradually enlarging abdominal mass, the presence of which was associated with amenorrhea. In addition, there were long-standing irregular nodular enlargements of the distal portions of the extremities.

The parents and the siblings (five) had no deformities. There was no history of similar enlargements occurring in any of the near relatives. The patient's infancy was uncomplicated. At the age of 5, bowing of the legs was noted, and a diagnosis of "rickets" was made. The bowing, however, was peculiarly asymmetric, involving mainly the left side. At the age of 9, the proximal epiphysis of the left leg was subjected to epiphyseal stimulation operation. The right leg had a corresponding epiphyseal arrest operation. At the age of 11, the distal epiphysis of the left forearm was subjected to epiphyseal stimulation. It is apparent, therefore, that the bone lesions were the first to occur. The first superficial nodule was noted on the dorsal surface of the distal phalanx of the right ring finger at the age of 14 years. It was followed rather quickly by other nodules, especially on the left hand and forearm and the left foot. The first nodule was bluish, compressible and soft, and others were of similar character, but the later prominent phalangeal enlargements were due to hard, fixed tissue.

Menstruation began at the age of 15, recurred at regular intervals and lasted four to five days. At the age of 19 years, or eight months before the patient entered the hospital, there was an abrupt cessation of menstruation. There was

gradual painless enlargement of the abdomen. This development had been known to the patient for a period of three to four months and was associated with the eight month period of amenorrhea.

The picture presented was that of a bowlegged white woman, 4 feet 7 inches (139.5 cm.) tall, with right dorsal scoliosis, compensatory lumbosacral scoliosis,



Fig. 1.—The photograph shows the deformity of the left shoulder and arm, the nodules of the digits, the genu varum and the attitude of scoliosis.

dropped left shoulder, short left arm and forearm, short left leg and multiple cutaneous and phalangeal nodules (fig. 1). The secondary sexual characteristics were well developed, although there was slight enlargement of the clitoris, as well as hypertrichosis of the legs. The head and the neck were normal.

Noteworthy physical findings were the orthopedic deformities and the abdominal mass.

The right arm was normally developed, and the finger tips reached the distal third of the femur. There were compressible bluish nodules on the dorsum of the wrist, the base of the thumb and the lateral aspect of the index finger at the distal end of the proximal phalanx, a fusiform swelling of the proximal phalanx and a cherry-like lesion of the nail bed of the ring finger. The nail of this finger was deformed. The veins of the hand and forearm were quite prominent, and two of the nodules at the wrist were situated along the course of a vein (fig. 2).

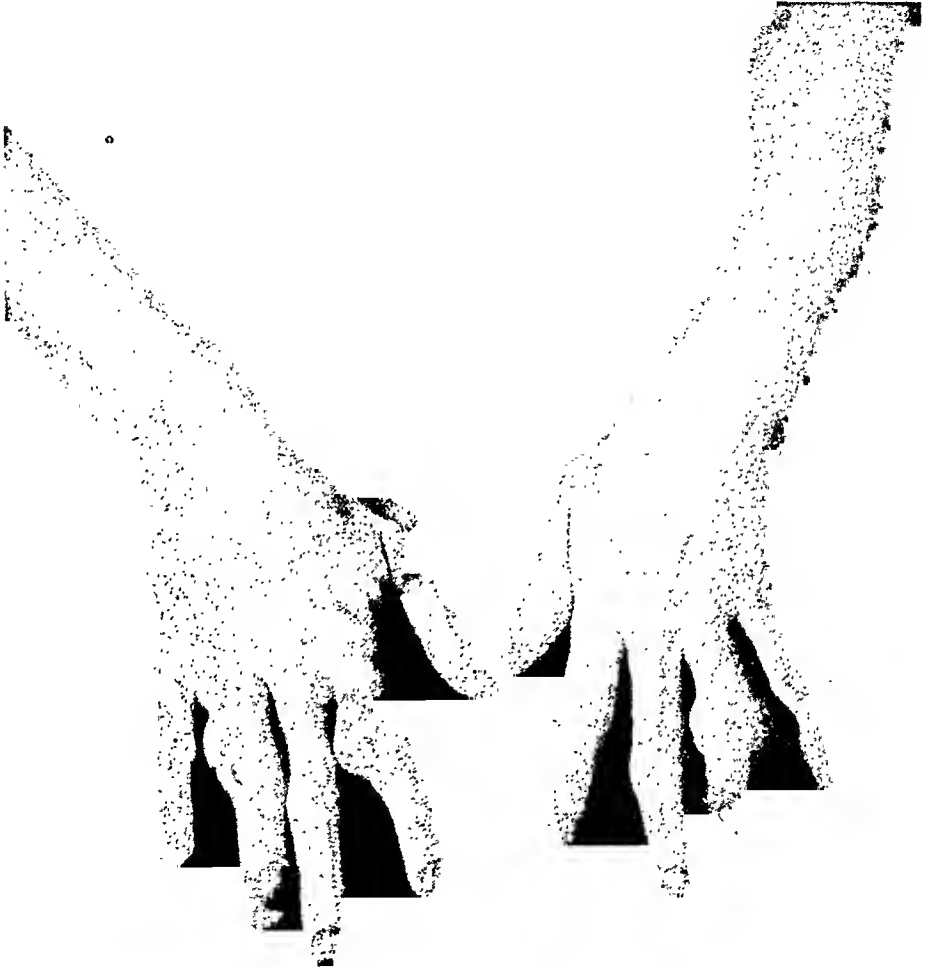


Fig. 2.—This infra-red photograph illustrates the vascular lesions of both hands and the prominent dilated venous system of the right hand and arm, along the course of which are two dark cutaneous nodules.

These nodules ranged from 1 to 2 cm. in diameter. The right leg presented marked genu varum, an old anterior tibial scar, with bluish red, soft nodules, 1.5 cm. in size, at the base of the second and third toes on the plantar aspect. There was dorsal crowding of the second toe, also flatfoot.

The left arm was quite short. Both the upper and the forearm were markedly shortened, so that the finger tips reached to the level of the femoral trochanter. The shoulder area presented a flattened deltoid region with the previously mentioned drop shoulder. There was a rather prominent valgus deformity of the elbow with marked lateral bowing of the forearm. The forearm was exceedingly short

and had a surgical scar at the distal ulnar epiphysis. The left hand was considerably smaller than the right and more prominently deformed. The largest of the multiple nodules was located at the proximal interphalangeal joint of the fourth finger. Other nodules were located at the distal end of the same finger and at the lateral margins of the other fingers, including the thumb. There were no palmar nodules in either hand. These nodules of the left hand were of mixed character. Some were bluish red and compressible, while others were quite hard and fixed to the bony substance of the finger.

The left femur was rather short and straight. The left trochanter was higher than the right and there was a tilt of the pelvis. The left leg revealed prominent lateral and anterior bowing. Multiple compressible soft bluish nodules were located along the anterior tibial line and prominently along the medial and lateral aspects of the ankle and heel. There was flatfoot of this extremity also.

Pelvic-rectal examination revealed a hard, smooth, nonfixed mass occupying the pelvis and extending up to a point 3 cm. above the umbilicus. It was the size of a five month pregnancy.

Roentgenograms of the right arm showed soft tissue nodules containing circumscribed radiopaque particles. There were no gross distinctive changes of the bony structures. The right foot showed radiolucent cystic areas in the great toe. There was a small radiolucent cystic area without disturbance of the cortex involving the subtrochanteric area of the femur. The left side presented multiple expansile cystic lesions of the clavicle, the scapula, the humerus, the radius, the ulna, the metacarpals and the phalanges. There was marked disturbance of the proximal end of the humerus and the proximal and distal portions of the radius (fig. 3A). The left wing of the ilium, the ischial rami and that part of the femur just below the trochanter exhibited similar radiolucent cystic areas (fig. 3B). The distal portion of the femur and the proximal portions of the tibia and the fibula were similarly involved. The phalanges of the left foot were the seats of punched-out cystic areas. There were a number of the calcified particles in the soft tissue nodules. Discrete radiolucent areas were noted in the ribs and in the right shoulder girdle. There was also the scoliosis mentioned. Roentgenograms of the pituitary area showed an irregular expanded outline of the sella turcica, suggesting a pituitary tumor.

There was moderate hypochromic normocytic anemia with considerable activity of the marrow. The blood sedimentation rate was 31 mm. in one hour and 61 mm. in two hours (Westergren). The serum proteins totaled 7 Gm., with albumin 5 Gm. and globulin 2 Gm., per hundred cubic centimeters. Acid phosphatase amounted to 4.6 and alkaline phosphatase to 13.8 King-Armstrong units. Blood calcium was 12.1 mg., phosphorus 3.2 mg., cholesterol 189.3 mg. and non-protein nitrogen 27.3 mg. per hundred cubic centimeters. The Friedman test for pregnancy gave a negative result.

The left ring finger was amputated at the metacarpophalangeal joint to relieve the discomfort caused by the large tumors. Several large tumors (hemangioma) were removed from the left plantar surface. These tumors had recently grown so large that a shoe could not be used, and locomotion was difficult.

Histologic examination of the bony tumors of the amputated left fourth finger showed a rather young myxomatous cartilaginous tissue. This tissue occupied the central portions of the phalanges to the exclusion of the bony substance. There were minute irregular spicules of bone about the periphery. In some instances clusters of large cartilaginous cells were found in large, round spaces. The picture was that of a mixed myxomatous and hyaline cartilaginous substance. Articular surfaces were intact.

The bluish cutaneous nodules and the deeply situated colorless tumors were examined, specimens being taken from different parts of the body. The bluish nodules consisted of numerous vascular spaces with a variable degree of cellularity. In most instances the pattern was that of a cavernous hemangioma; in other



Fig. 3.—*A*, roentgenogram showing the radiolucent expansile cystic lesions of the proximal portion of the humerus, of the clavicle and of the scapula. *B*, roentgenogram showing involvement of the left wing of the ilium, of the ischiorami and of the subtrochanteric areas of the femurs.

nodules, however, there was a cellular growth with sparse, minute, poorly defined vascular spaces. In the cellular areas, occasional mitoses were evident in the round or somewhat elongated, finely granular nuclei.

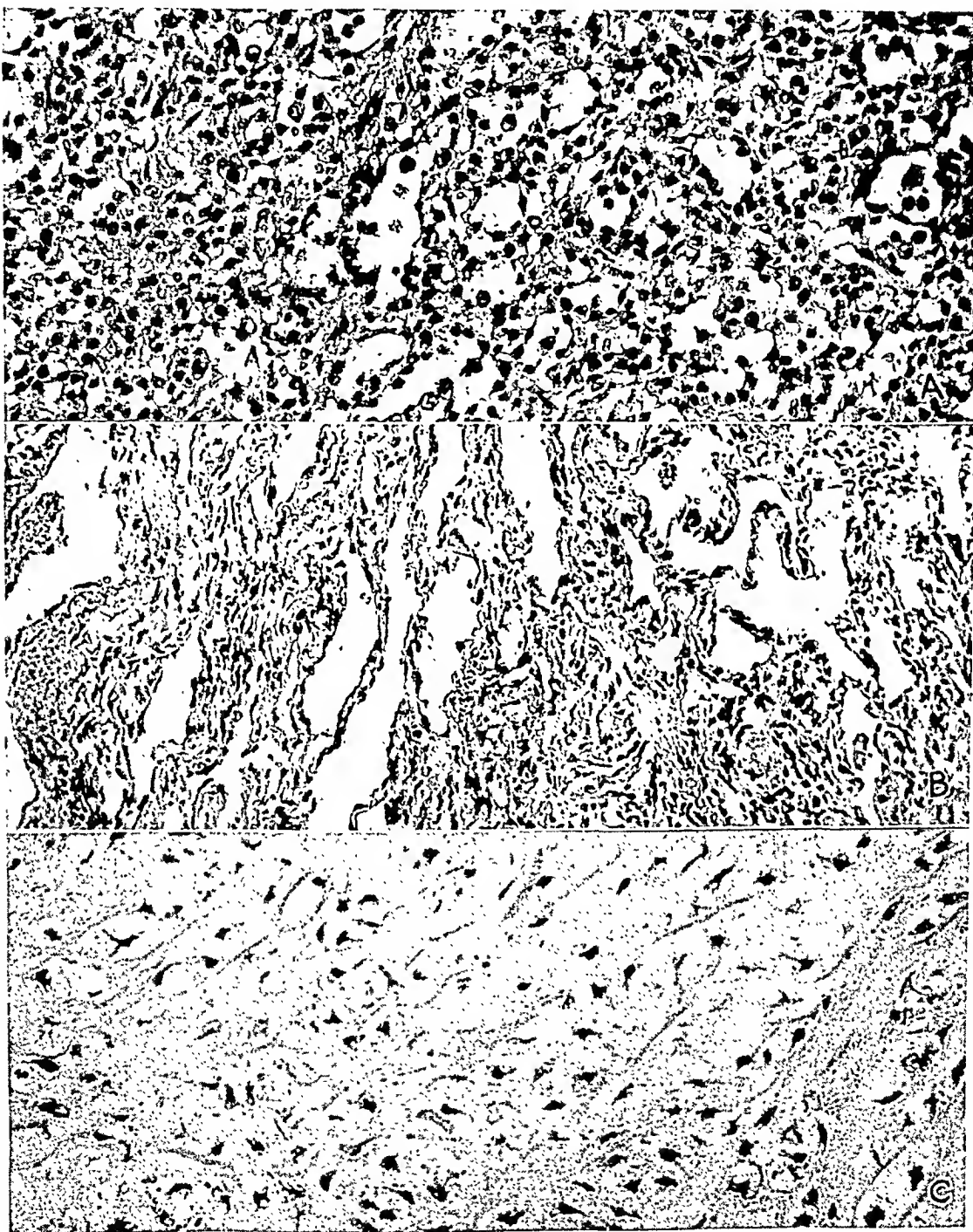


Fig. 4.—*A*, tumor. *B*, hemangioma of the skin. *C*, chondroma of the left fourth finger.

A large ovarian tumor of the left side was easily removed by laparotomy. There were no other intraperitoneal findings. It was a smooth mass weighing 1,900 Gm. and measuring 25 by 18.5 by 12 cm. Cut surfaces presented a grayish white, rather soft, bulging tissue arranged in lobular masses and large islands, separated by numerous but small cysts containing clear yellow limpid fluid. In some instances fibrous bands transected the cut surfaces, forming large lobulations. Some areas were deeply hemorrhagic and had yellowish foci of necrosis. Histologic sections presented a variegated surface, in which there was a pale, moist myxomatous type of stroma with large, slightly granular, lightly eosinophilic cells. These had poorly defined borders but round, finely granular nuclei. Some of the collections were associated with separation of the cell clusters into irregular small twisted columns. In other areas there was rather diffuse epithelium-like cell infiltration with little myxomatous stroma. The tumor has been studied by a number of pathologists. Five were of the opinion that it was an atypical granulosa cell tumor and not associated with endocrine function. One diagnosis was atypical arrhenoblastoma. Another was mesonephroma and another teratoma. The American Registry of Ovarian Tumors has classified the lesion as mesonephroma or teratoma. After removal of the tumor a simulated menstrual cycle was reestablished.

Two years after the removal of the ovarian tumor there was an insidious onset of weakness with loss of weight. Examination revealed a nontender, hard pelvic mass pressing on the rectum and fixing the vagina and uterus. Fever, abdominal pain, vomiting, and increase in size of the mass, with ascites, now came on rather quickly. In all 14,716 roentgens were given through various ports; but there was no response. At about the same time irregular cystic areas developed in the first, second and sixth left ribs and both shoulder girdles. The roentgenogram of the pituitary gland at this time showed an increase of the size of the sella turcica with destruction of the bony outline. A laparotomy was made on account of partial obstruction of the bowel, and the peritoneal surfaces were found to be covered with numerous irregular nodules of various sizes, microscopic examination of one of which showed peculiar myxomatous ovarian stroma resembling mucinous carcinoma. Some pathologists, however, held that it was a granulosa cell tumor, but the consensus was that it concerned a teratoid tumor. The symptoms progressively increased, and the patient died on July 8, 1946, three years after the removal of the left ovarian tumor. Unfortunately, permission to make an autopsy was not obtained.

COMMENT

It has been considered in the past that simultaneous occurrence of enchondroma and hemangioma is only coincidence. The tumors generally are not hereditary and are rarely found together, but each has been noted to occur with other defects. This case, however, presents threefold evidence of mesodermal dysplasia: multiple enchondroma, or dyschondroplasia; multiple hemangioma and a teratoid (mesodermal) tumor of the ovary. The family history does not contribute anything of significance. The history of rickets at the age of 5 years, if correct, would support Virchow's⁴ contention that the development of enchondromatosis represents misplaced immature cartilaginous rests brought about by improper osseous development. The rapid development of multiple en-

4. Virchow, cited by Maffucci, A.: *Movimento* 3:399 and 565, 1881.

chondroma suggests a rather prominently defective mesoderm; whether the finding is an anomaly of the vessels or a neoplasm has not been determined. In structure the tumors range from the usual cavernous hemangioma to a rather cellular angioblastic tumor.

SUMMARY

The twenty-second case of dyschondroplasia associated with hemangioma (Maffucci's syndrome) is recorded. The condition is a nonhereditary type of mesodermal dysplasia. The left fourth finger was amputated because of serious deformity, and a 1,900 Gm. teratoid ovarian tumor was removed, from which intra-abdominal metastases had developed.

Laboratory Methods and Technical Notes

ARGENTAFFIN CELLS OF THE HUMAN APPENDIX

A Comparative Study of the Results Obtained with Modified Schmorl and Masson Technics

A. LASKEY, B.S.

AND

J. GRECO, B.S.

BETHESDA, MD.

IN THE COURSE of Lillie's current investigations of the gastrointestinal mucins, it was found that the Heidenhain-Kulschitzky cells would reduce Schmorl's¹ ferric ferricyanide lipofuscin reagent. Since the method seemed, on preliminary trial, to be about as sensitive for the detection of these cells as the commonly used Masson silver method,² it was decided to compare these methods on a considerable series of human appendixes. While these studies were in progress, Gomori's³ paper appeared, in which he reported that the ferricyanides were reduced to ferrocyanides in the presence of ferric salts by the argentaffin or "enterochromaffin" cells.

In this comparative study, 464 formaldehyde-fixed appendixes cut at 5 microns were stained by the two technics, and the argentaffin cells were counted. Two appendixes had 4 cross sections per slide, 386 had 3, 67 had 2, and 9 had 1.

The technics used were:

Modified Schmorl technic:

1. Bring sections to distilled water.
2. Treat with equal parts of 1 per cent aqueous potassium ferricyanide and 1 per cent ferric chloride for 5 minutes.
3. Rinse in three changes of distilled water.
4. Stain for 1 minute in 1:5,000 new fuchsin (Color Index No. 678) in 1 per cent aqueous acetic acid.
5. Dehydrate in 95 per cent alcohol, absolute alcohol, alcohol-xylene and xylene, and mount as usual

From the Pathology Laboratory, Experimental Biology and Medicine Institute, National Institutes of Health.

1. Schmorl, G.: *Die pathologisch-histologischen Untersuchungsmethoden*, ed. 15, Leipzig, F. C. W. Vogel, 1928.

2. Lillie, R. D.: *Histopathologic Technic*, Philadelphia, The Blakiston Company, 1948, p. 102.

3. Gomori, G.: *Arch. Path.* 45:48, 1948.

Results: Argentaffin granules are stained greenish blue; cell nuclei, red.
Modified Masson argentaffin technic:

1. Bring sections to distilled water.
2. Stain in ammoniacal silver nitrate in daylight for 18 to 24 hours (until sections are dark amber). This solution is made as follows: To 4 cc. of strong ammonia (28 per cent NH_4OH) add 10 per cent aqueous silver nitrate (about 40 cc.) until solution remains faintly turbid on shaking.⁴
3. Rinse quickly in distilled water.
4. Tone for 5 minutes in Burtner's⁵ modified Ramón y Cajal gold toner. (The formula is 3 Gm. of ammonium thiocyanate, 3 Gm. of sodium thiosulfate and 100 cc. of 0.2 per cent gold chloride. Shake well before using.)
5. Rinse for 30 seconds in 5 per cent aqueous sodium thiosulfate.
6. Wash in running water for 2 minutes.
7. Counterstain as desired—safranín O (or others) dehydrate in acetones, acetone-xylene and xylene. Mount as usual.

Results: Argentaffin granules appear black; background, pink to brown; nuclei, red.

In comparing the two technics, adjacent cross sections were used. In such instances, often the number of argentaffin cells stained by either of the two methods exceeded considerably those shown by the other technic. A larger number of cells was demonstrated by the Masson technic in 261 cases and by the Schmorl technic in 179 cases; in 24 cases the same number of cells was demonstrated by each method. The average number of argentaffin cells per section with the modified Schmorl technic was 13.4. With the modified Masson technic the average was 14.7.

SUMMARY

Although both technics clearly differentiate the argentaffin cells, the Masson technic appears to have a slight advantage. However, it has the disadvantage of staining some extraneous material that can be mistaken for argentaffin cells. The modified Schmorl technic consistently stains the argentaffin cells greenish blue, but it shows inconsistency in the counterstaining, alternating from bright red to faint pink.

Hence, the two methods appear to have nearly equal value, and the Schmorl would be preferred when the relatively brief time required is an important consideration.

4. Lillie,² p. 187.

5. Burtner, H. J.: Unpublished data.

Books Received

HETERO-SPECIFIC ALTERATION THERAPY: A NEW TREATMENT FOR PULMONARY TUBERCULOSIS BASED ON SPECIFIC CELLULAR ALTERATION PRODUCED BY A MIXED AUTOLYSATE OF TYPHOID BACILLI AND GONOCOCCI. By Susumo Nukada, M.D., Ph.D., and Chieko Ryu, M.D., of the Nukada Institute and Sanatorium, Inage, Chiba-City, near Tokyo. Paper. Pp. 80, with 5 illustrations. Tokyo, Kyoto. The Japan Medical Publications Co., Ltd., 1948.

In 1924, Nukada and Matsuzaki reported that after rabbits had been vaccinated with isologous and various kinds of heterologous bacteria the antitoxic resistance of the heart showed remarkable variation even ten days after the last inoculation and that in animals vaccinated with a certain kind of heterobacteria the resistance of the heart was greater than that of nonvaccinated control animals. Subsequently, Nukada and his collaborators studied the fluctuations in the resistance or defensive power against bacterial infection of animals following vaccination (immunization) with different varieties of heterobacteria to note which produced the best protection. They found that a mixture of typhoid bacilli and gonococci produced the best "anti-tubercular" resistance. In the years from 1931 to 1937 Nukada and Ryu determined the minimal doses required to affect the course of experimental tuberculosis in rabbits and guinea pigs given large amounts of virulent human tubercle bacilli intravenously. Most of the treated animals survived longer than controls or those treated with colon bacillus or pneumococcus vaccine. Later an attempt was made to isolate the active principle, and it was found that "anti-tubercular" resistance was produced in guinea pigs by a mixture of an "autolysate" (autodigestion product) of typhoid bacilli and gonococci, to which they give the name "heterosate," and that this proved greater than the resistance produced with the bacilli themselves. The authors feel that the results produced with "heterosate" are due to an increase of resistance or defensive power against the tubercle bacilli developed as a "specific alteration" of the tissue cells. Tests were made on tuberculous patients (weekly subcutaneous injections), the doses being gradually increased, to avoid irritation, and improvement similar to that noted in animals resulted, particularly as pertained to reduction of temperature. Satisfactory results were noted in pulmonary tuberculosis.

The book is printed in English and presents tabulations of numerous experiments submitted in evidence. Although the data might be accepted as observational, it might be questioned whether the conclusions drawn warrant clinical therapeutic application since subjective findings may be so misleading. Scientifically, there are wide gaps in the reasoning applied to justify therapeutic conclusions. The "heterosate" prepared from heat-killed organisms (53 C. for one hour) and containing phenol can hardly be called an autolysate in the strict definition of this phenomenon. There is also some confusion as to its effectiveness as a preventive or a therapeutic agent. Preventive tests proved a lesser incidence of tuberculosis in nurses (44 persons), and therapeutic tests made in 932 cases of pulmonary tuberculosis gave "very satisfactory" results. The injection of "heterosate" never produced secondary ill effects. It is proposed to call this method of treatment of pulmonary tuberculosis "hetero-specific alteration therapy" or "heterosate therapy," terms based on the conception that a cellular

alteration is produced by subcutaneous injections of a mixed autolysate (term questioned by the reviewer) of typhoid bacilli and gonococci ("heterosate"), whereby the resisting or defensive power of the tissue cells is increased specifically against tubercle bacilli and, in consequence of this, the cure of the disease promoted. The book should be read and evaluated critically by those interested.

CANCRO: CARCINOGENESE, CARCINORESISTENCIA, CARCINOINIBICAO. By Michel Mosinger, *Arquivos de anatomia patológica, patologia correlativa e neuro-ergonologia*, volume 33—1946-1947 (Tome 1). Pp. 287. Coimbra, Portugal: Coimbra Editora, Limitada, 1947.

This entire number is written (in French and Portuguese) by Prof. Dr. Michel Mosinger, director of the Institute of Pathologic Anatomy of the University of Coimbra. It contains summaries and conclusions of experiments performed since 1936 by the author and his associates, together with brief bibliographies, references to the original publications, and 252 figures, mostly photomicrographs. The experimental work includes, among others, studies of guinea pigs, rats and mice treated with estrogens and carcinogens.

CORRECTION

In the article by Dr. George Gomori, "The Chemical Character of the Enterochromaffin Cells," in the January issue (*Arch. Path.* 45:48, 1948), in the last paragraph on page 49, "strong solution of iodine U.S.P." was substituted for "Lugol's solution," which as understood by histologists is the Gram modification, containing 0.33 per cent iodine, while strong solution of iodine U.S.P. contains 5 per cent iodine.

DIFFUSE ANGIECTASIS OF THE CEREBRAL MENINGES OF THE NEWBORN INFANT

Report of Three Cases

EDITH L. POTTER, M.D., Ph.D.
CHICAGO

A LOCALIZED increase in the number, the size or the tortuosity of the vessels of the meninges covering the cerebrum or the cerebellum has been described by numerous observers. The increases have been variously situated, and in many instances the vessels have extended into the brain beneath the areas of meningeal involvement. They have often been associated with abnormalities of blood vessels in other locations, especially the face and the retina.

An extensive search of the literature has failed to reveal descriptions of vascular abnormalities generalized over the surface of the brain in any age group similar to those to be described in the present report. In a few previously reported cases the entire meningeal area exposed at operation was abnormal, and it may be possible that the lesion involved more of the meninges than was apparent to the surgeon. Against this possibility is the fact that in most instances definite localizing symptoms were present. Such a case was reported by Bailey,¹ and his description of the localized tumor of his patient applies almost exactly to the appearance of the more widespread lesions found at postmortem examination in the present cases. Bailey stated that "all over the exposed cortex the vessels were greatly dilated. In the temporal and parietal regions were tangled masses of small vessels and deep in the temporal region were more tortuous vessels which could not be exposed."

As far as I have been able to determine, tumors arising from meningeal vessels or malformations of meningeal vessels which might be interpreted as angioma or other types of vascular tumor have never been described as observed in fetuses or young infants.

The present report concerns 3 newborn infants whose meningeal vessels showed remarkable changes of gross appearance. In each instance there was also an abnormality of the heart.

From the Department of Obstetrics and Gynecology of the University of Chicago, and the Chicago Lying-In Hospital.

1. Bailey, P.: *Intracranial Tumors*, Springfield, Ill., Charles C Thomas, Publisher, 1933.

The vessels of the meninges of 2 infants were almost identical in appearance (fig. 1). Over the entire surface of the cerebrum and the cerebellum these vessels were of fairly uniform size and exhibited a great increase in number and tortuosity. They formed a scroll-like pattern, and their many convolutions produced a generalized tangled mass over the exterior of the brain. They had no connection with the vessels of the brain or those of the dura mater, and all were confined to the leptomeninges. The membrane holding the vessels together was fragile and easily torn. When this membrane and its associated blood vessels were removed, the underlying surface of the brain was smooth, white and normal in appearance. The histologic structure of the brain was normal.

These meningeal blood vessels had the structure of capillaries and terminal veins. Many vessels were composed of a single layer of endothelial cells, while others were made up of endothelium surrounded by a few muscle cells. In the third infant the appearance of the meningeal vessels was somewhat different, although here, too, a scroll-like tortuosity was the principal abnormality (figs. 4 and 5). The increase of the total number of vessels was somewhat less striking than in the other infants, and the proximal portions were of considerably greater caliber than were the distal branches. Greater irregularity of the size of the venous channels was thus produced, and nowhere was there such great concentration of vessels as was present throughout the meninges of the other infants.

Each of the 3 infants had an associated abnormality of the heart. In 2 it consisted of generalized hypertrophy. The size of the chambers was abnormally great, and the muscle was increased in thickness, but the valves and the great vessels leading from the heart were normal (figs. 2 and 3). No glycogen could be demonstrated in the muscle cells. The third infant had only slight enlargement of the heart, but the left innominate vein entered the left atrium instead of joining the right innominate vein to form the superior vena cava (fig. 6). This infant also had an abnormality of the liver consisting of the presence of numerous superficial veins, lying immediately beneath the capsule, a diffuse dilatation of the veins in the center of the lobules and a moderate increase in periportal connective tissue.

It seems more than coincidence that this abnormality of cerebral vessels has been observed on three occasions in infants who also had abnormalities of cardiac development. If the changes in the meningeal vessels are related to cardiac hypertrophy, it is interesting to speculate on cause and effect. Could it be that the heart hypertrophied in order to supply the increased vascular bed in the meninges, or could the hypertrophy of the heart have caused a sufficient increase in cardiac

output and pressure to be responsible for hyperdevelopment of the meningeal vessels? In either instance one would expect the arterial system to be as greatly or more greatly involved than the venous, and in all 3 infants the arteries in the body appeared normal.

It is also interesting to speculate on the symptoms that would have been produced by this great increase in meningeal vessels had the infants survived. The fact that it is not a condition recognized in later life suggests that it may be incompatible with continued existence and that all those affected die in early infancy.

REPORT OF CASES

CASE 1.—The mother was a 23 year old primipara whose pregnancy was normal and who was delivered at term by low forceps after twenty-four hours of labor. The fetal heart tones disappeared during labor, and the infant was dead at birth. A twin weighing 935 Gm. was born ten minutes after the first infant. Maceration was extreme, and no abnormalities could be made out.

The first twin was a boy weighing 3,832 Gm. and measuring 56.5 cm. in total length. Moderate generalized edema which was most severe in the region of the ears and the eyelids was present. The tongue protruded slightly from the open mouth. No fluid was present in the chest, but about 50 cc. was found in the abdominal cavity.

The most notable changes were in the meninges, the heart and the thyroid gland. The meninges covering the entire brain contained vessels which were greatly increased in length and number. The surface was so covered with the innumerable loops and coils of the venous channels that the underlying brain was almost invisible (fig. 1). The vessels were of approximately uniform caliber, were distended with blood and in no place penetrated the brain. In appearance they were similar to capillaries and thin-walled veins; many of the vessels were made up of a single layer of cells. No vessels having the structure of arteries were found in the involved areas.

The heart was remarkably enlarged and weighed 68 Gm. (fig. 2). All its parts were increased in size as a result of hypertrophy of the cardiac muscle and enlargement of the chambers. The valves, the great vessels and the septums were normal. The histologic structure of the cardiac muscle was normal, and glycogen could not be demonstrated in the cells.

The thyroid gland was remarkably increased in size and weighed 6.5 Gm. The vessels were distended with blood and were conspicuous, although no actual increase in number appeared to exist. The acini contained no colloid, and the lining cells were partially desquamated. In spite of the increased size of the gland, no changes which were clearly pathologic could be made out.

The lungs were hypoplastic as a result of being directly compressed by the enlarged heart, and their combined weight was 20 Gm.

The remaining organs were within normal limits in size and histologic structure. The thymus was somewhat smaller than usual, and the liver contained larger and more numerous areas of erythropoiesis than are ordinarily present; similar findings, however, may occasionally be present in otherwise normal infants. The majority of the blood vessels throughout the body were distended with blood.

CASE 2.—The mother was a 40 year old Negro woman who had had seven normal pregnancies. Her antepartum course was uneventful. She went into labor spontaneously and was delivered by breech extraction after a total labor of nine

hours. Membranes had ruptured about twenty-four hours before the onset of labor. The infant breathed immediately after birth but subsequently became dyspneic and cyanotic. These symptoms became progressively worse, and death occurred twenty-five hours after birth.

The infant was a girl, weighing 5,810 Gm. and measuring 60 cm. in length. No external abnormalities were visible, and much of the increase in weight was due to an excessive deposit of subcutaneous adipose tissue.



Fig. 1 (case 1).—Surface of the brain, showing angiectatic capillaries and veins in the leptomeninges. The white areas are artefacts caused by tearing of the arachnoid membrane during removal of the brain.

The meninges and the heart were remarkably similar to those described in case 1. The vessels of the meninges were excessive in number and length and formed a tangled mass which was generalized over the entire surface of the brain. They did not penetrate the surface of the brain but remained confined to the meninges. The appearance was similar to that reproduced in figure 1.



Fig. 2 (case 1).—Opened thoracic and abdominal cavities showing the extremely hypertrophied heart almost completely filling the thoracic space.



Fig. 3 (case 2).—Thoracic cavity showing extreme cardiac hypertrophy.

The heart also was similar to that of case 1 except that the apex was more pointed and the atriums were not proportionately as greatly increased (fig. 3). The ventricular and atrial walls were thickened, and the chambers were enlarged. The valves and the great vessels were normal. The weight was 71 Gm.



Fig. 4 (case 3).—Superior surfaces of the cerebral hemispheres, showing dilated angiectatic veins and capillaries in leptomeninges.

The lungs appeared to be normally developed and were increased in weight as a result of pneumonia. The pneumonic process may have been caused by the aspiration of amniotic fluid following premature antepartum rupture of the membranes. The lungs weighed 120 Gm.

The thyroid gland was slightly enlarged and weighed 3.5 Gm. The vessels were greatly distended, no colloid was present and the cells lining the acini were partially desquamated.

Mild erythropoiesis was present in the liver. The remaining organs showed nothing of note except extreme congestion.



Fig. 5 (case 3).—Inferior surfaces of the cerebral hemispheres and the cerebellum, showing tortuous, scroll-like vessels similar to those on the superior surfaces.

CASE 3.—The mother was a 25 year old woman who had had one normal pregnancy. She had symptoms of mild toxemia and was admitted to the hospital four times during pregnancy because of abdominal pains which were thought to threaten early termination of pregnancy. She went into labor spontaneously at thirty-eight weeks and was delivered by low forceps after nineteen hours of labor.



Fig. 6 (case 3).—Opened body cavities showing (1) the left superior vena cava entering the left atrium and (2) dilated, abnormally placed veins on the surface of the liver.

The infant was cyanotic at birth, and respiration was established with difficulty. The cyanosis continued, respiration was of poor quality and death occurred after thirty-two hours.

The infant was a boy weighing 2,900 Gm. and measuring 48 cm. in length. As in the other 2 infants, the meninges and the heart were the site of the principal lesions. The vessels of the meninges, however, were somewhat different; the principal vessels were increased in caliber, and the smaller vessels, although tortuous and circinoid, were involved to a somewhat lesser degree than those of the other infants. (Compare figures 4 and 5 with figure 1.) The brain substance was normal.

The heart was stated to have been enlarged, but it was not weighed, and the increase in size does not seem to have been appreciable. The left innominate vein, instead of uniting with the right innominate vein to form the superior vena cava, entered the lateral wall of the left atrium anterior to the pulmonary veins. The membrane covering the foramen ovale was unusually thick and was herniated into the right atrium. The heart was otherwise normal.

The liver of this infant was also abnormal and was covered superficially with prominent, dilated veins. The central veins within the liver were dilated and unusually prominent. Connective tissue was present in excessive amounts in the periportal areas and in lesser amounts in irregular areas between the lobules.

SUMMARY

Three infants suffered an abnormality of development in the meningeal vessels over the entire surface of the brain. The vessels were similar to those found in localized lesions in older persons which have been described under the name of meningeal angioma. The infants described in the present report had a generalized involvement of all meningeal vessels producing a diffuse angiectasis in the cranial meninges. A cardiac abnormality was also present in each infant.

SPECIFIC GRAVITY OF THE BLOOD CORPUSCLE

Its Possible Significance in Atherosclerosis

ISRAEL GORDON, M.D., M.R.C.P., D.P.H.

ILFORD, ESSEX, ENGLAND

IN A previous communication I¹ suggested that the lipophage, because of its high content of fatty esters, would have a lower specific gravity than the other cells of the blood and thus be the first to enter the peripheral zone of the blood stream when the velocity diminished, and so be in a situation to enter the intima of the elastic arteries, causing the primary lesion in atheroma. In common with the authors of all current textbooks of pathology I accepted rather uncritically the hypothesis that cells of the blood of low specific gravity did leave the axial stream first because of their specific gravity. Schklarewsky² first suggested this, but no one except Hamilton³ has apparently ever considered why it should be so and Hamilton's 63 year old explanation is certainly wrong, as it is based on the assumption that the erythrocytes have a specific gravity approximating that of the plasma and that the white corpuscles are lighter than the plasma. In fact, Fahreus⁴ and his pupil Vejlens⁵ have demonstrated clearly that the position of a cell in the blood stream may depend on its size, the larger cells being carried more centrally in the vessel than the smaller ones. From this demonstration they have decided that the specific gravity can be of no importance whatsoever, that Schklarewsky, being a Russian working in Germany and not understanding German properly, did not really mean that specific gravity was important when he said so, and that the leukocytes in his experiments left the axial stream first because in the frog leukocytes are smaller than erythrocytes. In man, of course, leukocytes are larger than erythrocytes, but when the current is slow enough the erythrocytes agglutinate and the clumps are then larger than the individual leukocytes.

While agreeing that size is one factor influencing the place of a corpuscle in the blood stream I feel that, for reasons to be discussed,

From the Public Health Offices.

1. Gordon, I.: Arch. Path. **44**:247, 1947.

2. Schklarewsky, A.: Arch. f. d. ges. Physiol. **1**:602 and 657, 1868.

3. Hamilton, D. J.: J. Physiol. **5**:66, 1884-1885.

4. Fahreus, R.: Physiol. Rev. **9**:241, 1929.

5. Vejlens, G.: Acta path. et microbiol. Scandinav., 1938, supp. 33.

it is not the only factor or even the main one. If the corpuscles in a vessel were confined to a cylinder two-thirds the diameter of the vessel they would form a solid mass. This does not happen; so some force or forces must keep the corpuscles apart as well as draw them to the center. The story about Schklarewsky is rather weak, for Thoma,⁶ a German, worked with Schklarewsky in the laboratory of Helmholtz, and Thoma, in his textbook, has been emphatic about the influence of specific gravity, and he could not have had trouble with the language. I feel that all parties to the discussion have ignored a fundamental fact, that the place of a corpuscle in the blood stream must also depend on its contacts with the erythrocytes.

In the male 47 per cent, in the female 42 per cent, of the volume of the blood consists of red blood corpuscles; thus the space between erythrocytes is not much greater than the volume of the erythrocyte itself. Furthermore, in the peripheral regions, especially, these corpuscles are traveling at different velocities. Except for the lightest gases, fluids in tubes flow either in laminar or in turbulent fashion. In the smaller vessels the flow is undoubtedly laminar, as this has been demonstrated cinematographically by Knisley and associates.⁷ The type of flow in the aorta and the larger elastic arteries has not been determined, but even if it is turbulent, there is a steep gradient of velocities near the periphery. The type of distribution of velocities in turbulent flow may be illustrated as in figure 1 *A*; and laminar flow in a fluid containing a large number of corpuscles is, according to Vejlens,⁸ somewhat similar, the paraboloid shape of laminar flow in water being blunted as in figure 1 *B*. In either case there is a thin boundary layer where the velocity steeply falls to zero; inside this layer in laminar flow the blood moves regularly in laminae, the nearer the center the more swiftly; in turbulent flow it proceeds in eddies. In laminar flow there is probably little bumping of one red corpuscle with another, for the erythrocytes (Knisley and associates⁷) also flow in laminae, each one corpuscle thick. A corpuscle larger than an erythrocyte, however, would be subject to many collisions.

EFFECT OF CONDITIONS IN THE SMALLEST VESSELS

According to Fahreus⁴ and Vejlens,⁸ in what they termed the "para-capillary" vessels the leukocytes, being larger than the erythrocytes, flow nearer the center. However, when the velocity is reduced, or in certain other circumstances, the red cells clump together, and these aggregates, being larger than the leukocytes, flow in the center. The

6. Thoma, R.: *Text Book of General Pathology and Pathological Anatomy*, London, Adam & Charles Black, 1896.

7. Knisley, M. H.; Block, E. H.; Eliot, T. S., and Warner, L.: *Science* **106**: 431, 1947.

authors then gave this changing of position as sufficient reason why the leukocytes begin to appear in the hitherto cell-free peripheral zone. It is difficult to see their reasoning, for the axial zone is still the same size and contains no more cells; in fact, Fahreus stated that the blood corpuscles are diluted in these paracapillary vessels, and thus there is even more room for them in the axial stream. It is here that another mechanism must be considered. The erythrocyte clumps (or sludges as Knisley called them) traveling more centrally also thus travel more quickly. The red cells, being nearly half the volume of the blood, frequently overtake the leukocyte on its inner side and collide with it. The forces concerned are, in respect of the bumping corpuscle, its kinetic energy and, in respect of the bumped corpuscle, its inertia. Kinetic energy is expressed as $\frac{1}{2} m V^2$ (m = mass and V = velocity—in this case the difference between the velocity of the leukocyte and that of the agglutinate). The inertia of the bumped

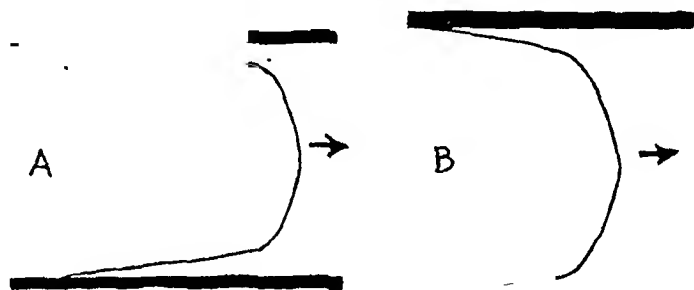


Fig. 1.—*A*, diagrammatic representation of the distribution of the differing velocities in a blood vessel with turbulent flow; *B*, that in a vessel with laminar flow.

corpuscle also depends on its mass. Now mass is the product of volume and specific gravity; the volume of the red cell aggregate is greater than the volume of the leukocyte; its specific gravity is greater (the specific gravity of the red blood cell is 1.094; that of the leukocyte, 1.061, according to Vejens⁵). The leukocyte that is not on the edge of the axial stream would in its turn bump the more slowly flowing erythrocyte masses on its outer side, but would have a smaller effect in dislodging them, as its mass is so much less, and, on receiving impacts on its inner side when space is available between red cell clumps, would be bounced out. The reaction of the leukocyte would, however, be the resultant of two forces; it would be bounced out only when the colliding forces are greater than the force tending to draw it toward the center. Thus the leukocyte, because of its smaller mass, would tend to be bounced out of the axial into the peripheral zone. In this restricted sense, then, specific gravity is of importance, as it is a factor in the determination of mass.

EFFECT OF CONDITIONS IN THE ELASTIC ARTERIES

Although Knisley and associates⁷ have found sludged blood in hypertension and arteriosclerotic heart disease, it would be wrong to assume that sludged blood is always present in the aorta when that vessel is atheromatous. If the blood is sludged, the lipophages would be bounced out by the heavier erythrocyte masses when the indrawing forces are lessened, as described earlier.

That the lipophage has a lower specific gravity than the polymorphonuclear leukocyte or the lymphocyte is almost certain. The specific gravity of the rabbit polymorphonuclear leukocyte is about 1.061; that of cholesterol is 1.046, and that of its esters would be even less. I have been unable to obtain the specific gravity of pure cholesterol esters, but there is no reason to assume that the lipophage is full of pure esters only; it probably contains other fat-soluble substances as well, and in fact, if one is to judge by the fat content of the atheromatous intima (Page⁸), it probably contains a considerable proportion of neutral fat. An impure mixture of cholesterol esters is anhydrous wool fat, the specific gravity of which is as low as 0.0940.⁹ The only correct answer will be, when found, the specific gravity of the lipophage itself, but it must be less than that of the polymorphonuclear leukocyte and is perhaps even less than that of plasma (1.028).

The following argument can be concerned only with lipophages in the boundary zone of the axial stream. Leukocytes nearer the center will obviously be out of the aorta before much change in their position can be effected, and since the stream is probably turbulent, the argument will not apply. The size of the lipophage from which the mass is deduced is of great importance, for in the case now considered the erythrocytes flow individually, i. e., not in sludges, and thus have a much reduced mass. Anitschkow¹⁰ stated that the lipophages may vary in size from that of a lymphocyte to that of a large monocyte, and Leary¹¹ has pointed out that they are small in the lung capillaries and tend to become larger in the tissues. At times they are big, and Beard and Rous¹² have described Kupffer cells, from which they are derived, growing to the fantastic size of 540 microns. This increase in size increases the mass, and so also the inertia, but with increase in size it can be shown that there is a corresponding increase of packets of kinetic energy striking the cell. Those lipophages of the size of polymorpho-

8. Page, I. H.: *Biol. Symposia* 11:43, 1945.

9. *Codex Medicamentarius Gallicus: Pharmacopée Française*, ed. 6, Rennes, Imprimeries Oberthur, 1937, vol. 2.

10. Anitschkow, N., in Cowdry, E. V.: *Arteriosclerosis*, New York, The Macmillan Company, 1933.

11. Leary, T.: *Arch. Path.* 32:507, 1941.

12. Beard, J. W., and Rous, P.: *J. Exper. Med.* 59:593, 1931.

nuclear leukocytes or less would have less inertia than these cells and so be more easily displaced out. It is difficult to see how any leukocytes can be drawn into the center as Fahreus⁴ described, as they are continually being overtaken by erythrocytes on their inner sides, these erythrocytes having a greater velocity.

It is in the large elastic vessels that the factor of diastole becomes important, for during this time, which is longer than systole, the velocity of the blood must be considerably reduced; in fact in places in the aorta the blood probably reverses its direction. This marked slackening of velocity, less noticeable in the arteries further from the heart, means a reduction in the force drawing the cells toward the center of the vessel. However, the velocity of the corpuscles in the blood would not be reduced to the same extent as that of the plasma, for the corpuscles possess inertia and for a small fraction of time would tend to continue to move in their original direction, until the next systolic impulse moves both corpuscles and plasma forward again. Thus the velocity of the blood stream, the force tending to pull corpuscles in, is reduced more than the velocity of the corpuscles, the impinging of one on the other being the force tending to drive the larger and lighter corpuscles out.

EFFECT OF THE SIZE OF CORPUSCLES

Under this head three principles must be taken into account:

1. The larger the corpuscle the greater is its surface area, and so the larger is the surface exposed to impinging erythrocytes. However, this larger surface does not make up for the increase in mass. If one corpuscle is twice the diameter of another, its surface will be increased four times, but its volume, and therefore mass, will be increased eight times.

2. The larger the corpuscle the greater will be the velocity of erythrocytes colliding with its surface nearer the center of the vessel. As the kinetic energy varies as V^2 , and as V is the difference in velocity between the leukocyte and the bumping erythrocyte, the larger leukocyte will have packets of much greater energy tending to drive it out.

3. The larger the corpuscle the more often it will be bumped on its inner side, for these erythrocytes are traveling with greater velocity and will thus be passing by more frequently.

The sum of 1, 2 and 3 means that, although larger and thus of greater mass, the larger lipophages quite possibly would receive vastly increased complements of energy tending to drive them out. Finally, the larger lipophages, because of their greater mass and hence greater kinetic energy, would bump away more easily the erythrocytes that they themselves overtake.

It would be interesting to clothe the skeleton of this hypothesis with details of actual velocities, and in fact equations are available to find the velocity of any particle at a given distance from the center of a tube. It is considered that the type of flow in the aorta is turbulent, for the following reasons: 1. The diameter is relatively large. 2. The velocity is relatively great. 3. The flow is intermittent. 4. Large tributaries would tend to disturb any even flow. The equation for turbulent flow given by Kármán,¹³ cited by Vejlens,⁵ is

$$w = W_{max} [1 - (\frac{y}{r})^2]^{1/7}$$

where

w = velocity of particle

W_{max} = maximum velocity of fluid

y = distance of particle from center of tube

r = radius of tube

Immediately, however, it is apparent that in the aorta there are factors introducing a large margin of error into the use of this equation: 1. The velocity in the aorta differs from systole to diastole. 2. The diameter of the aorta is greater in systole than in diastole. 3. The region in the aorta where the axial zone passes into the peripheral zone, i. e., where these collisions in which one is interested occur, is unknown. Vejlens⁵ stated that the peripheral zone is always 1 red cell diameter wide, irrespective of velocity or size of vessel. Sandison¹⁴ expressed the belief that the peripheral zone is larger the swifter the stream. Perhaps it varies with systole and diastole. For the purpose of this illustration it is considered as 7 microns. 4. The velocity of each corpuscle is considered to be that of the stream of plasma corresponding with the center of the corpuscle, an assumption by no means necessarily true. 5. The diameter of the aorta varies with age and so also the velocity in the vessel.

Accepting the premise that if one considers the aorta as a rigid tube with a stream of constant velocity one is inviting a large margin of error except perhaps for a short period in the cardiac cycle when that diameter and velocity obtain, one may indicate the velocity of the corpuscles concerned as shown in figure 2 for each cell.

For convenience, erythrocytes 7 microns in diameter are shown as circular. Cell A, 12 microns in diameter, is a polymorphonuclear leukocyte. Cell B, 20 microns in diameter, is a lipophage. The remaining cells are erythrocytes. Velocities are expressed in millimeters per second to the nearest tenth of a millimeter. The maximum velocity

13. Kármán, V.: Ztschr. f. ang. Math. u. Mechanik. 1:233, 1921; cited by Vejlens.⁵

14. Sandison, J. C.: Anat. Rec. 54:105, 1932.

of the aortic blood stream is taken to be 300 mm. per second (Cowdry¹⁵), and the diameter of the aorta to be the average at age 50 found by Kaufman and Aschoff, cited by Krafka,¹⁶ i. e., 22 mm.

It is apparent that erythrocyte 2 is advancing on polymorphonuclear cell A with a velocity of 5.5 mm. per second (131.9—126.4). Erythrocyte 6 is advancing on lipophage B with a velocity of 7 mm. per second (138.4—131.4). Since kinetic energy varies as V^2 , corpuscle B receives an impulse about 1.6 times that received by corpuscle A with respect to the erythrocytes mentioned. Also, erythrocyte 6 is advancing on corpuscle B with velocity 1.3 times as great as that with which E 2 is advancing on A; therefore B will receive 1.3 times the number of impulses—presuming erythrocytes follow each other in series. It will receive in addition the contacts of erythrocyte 5, which, however, in this case will be of relatively low kinetic energy. Corpuscle A itself bumps erythrocyte 7, and corpuscle B bumps erythro-

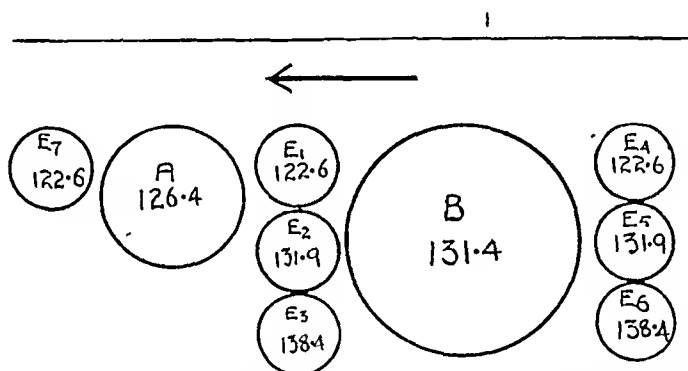


Fig. 2.—Blood stream velocities of a polymorphonuclear leukocyte (A), a lipophage (B) and erythrocytes (E 1 to 6).

cyte 1, but corpuscle B, because of its larger mass and greater velocity, has at least twenty times the kinetic energy of polymorphonuclear cell A with respect to the erythrocytes concerned; therefore it pushes the erythrocyte aside more easily. All these points demonstrate that the larger the cell the more easily it is extruded. It must be remembered also that cell B has approximately three times the surface area of cell A, allowing three times the space for collisions. Against all these factors, however, it has nearly five times the inertia.

At first it may not seem significant that an erythrocyte should overtake and collide with another corpuscle at a velocity of 5 mm. per second. However, for such a small body this is a great velocity; using common methods of analogy one finds that the erythrocyte 7

15. Cowdry, E. V., in *Arteriosclerosis*, New York, The Macmillan Company, 1933.

16. Krafka, J.: *Arch. Path.* 20:81, 1935.

microns in diameter can pass 500 polymorphonuclears 10 microns in diameter laid side by side in one second, and a bumped corpuscle can suffer several hundred impacts in the same period. However, too much stress should not be placed on the data given; in view of the mathematical and hydrodynamic difficulties of the problem they can be considered only as illustrative. As there is about 1 leukocyte to every 600 erythrocytes, it is hardly necessary to consider the effect of leukocytes colliding with erythrocytes on the distribution of erythrocytes and quite unnecessary to consider the effect of one leukocyte colliding with another.

It is apparent that in the phenomena so far described size of corpuscle is of far more importance than the relatively small differences in specific gravity. It appears, in fact, in the light of present knowledge that only when the influence of gravity is brought to bear will corpuscles of a specific gravity greater than plasma act much differently from corpuscles of a specific gravity less than plasma, as some well filled lipophages may well be. The effect of gravity will obviously be to make the former settle and the latter rise. When the current in a small vessel that is nearly horizontal is slowed, the erythrocytes tend to settle; this phenomenon has been described and photographed by Knisley and associates.⁷ Presumably, also, corpuscles of a specific gravity less than that of plasma will in the same circumstances rise. The velocity in the aorta is greatly reduced in diastole; in the peripheral zone of the stream into which large cells are bounced it is much slower. The effects of several diastoles will be cumulative. Whenever the walls of the aorta are out of the vertical, this process of rising of the light particles and falling of the heavy ones will tend to bring the corpuscles into contact with the walls if the current is slow enough. Gravity will thus exert its action in that narrow region a few microns from the intima. Lipophages will tend to rise and stick to the intima, especially during diastole. It is true that erythrocytes will settle on the intima below as well, although there will not be the same tendency for them to be pushed into the peripheral zone; but this is of little consequence, as they will not stick. Beard and Rous¹² have described the stickiness of Kupffer cells in culture as far surpassing that of any other cells, including polymorphonuclear leukocytes.

It is apparent that macrophages containing many substances, occurring naturally or experimentally, can circulate in the blood. Leary¹¹ mentioned silica and silicates, carbon, magnesium dioxide, lapis lazuli, mercuric sulfide, colloidal dyes, bacteria and blood pigments. These cells, with the exceptions described later, never appear in the intima; only cells containing lipids do so. Leary suggested that this difference is due to chemotaxis. I suggest it is due to specific gravity. The

exceptions are the macromolecular substances of Hueper,¹⁷ and it would be interesting if one could be informed of their specific gravity, remembering, however, that injection of these substances may well change the suspension stability of the blood, causing aggregation (or sludging) of erythrocytes, thus enormously increasing the mass of these cells as compared with leukocytes.

It is also necessary to call attention to the report of Katz and Dauber¹⁸ that in cholesterol-fed animals the intimal capillaries of the aorta were crammed with lipophages. Why should this be so, unless the peripheral stream of the aorta contained chiefly lipophages? Chemotaxis here seems unnecessarily speculative. Why should the peripheral stream then be full of lipophages and not full of the more numerous polymorphonuclear leukocytes? Again it seems that size and specific gravity appear the only satisfactory answers.

Unfortunately, the problem is by no means as simple as I have demonstrated. It is in fact of the greatest complexity, and a full analysis of all the factors concerned will require the application of the finest mathematical minds to a difficult problem of hydrodynamics. In considering the reaction of one cell with another it will be necessary to include the effect of viscosity, the effect of the shape and the lie of erythrocytes at the time of impact, the differential effect of the impact according to the position of the erythrocyte on the circumference of the leukocyte (i. e., the more tangential the blow the less the bumped cell will be pushed forward and the more it will be pushed aside) and the variations in size of the vessel and in velocity of flow with the cardiac cycle. Also to be considered are the kinetic energy of the particles of plasma themselves as they strike the corpuscle, and the electromagnetic attraction and repulsion of corpuscles, one with another. Finally, there may be other factors, as yet undescribed, that may influence the position of corpuscles in the blood stream, and dogmatism such as Fahrens⁴ and Vejlen⁵ have shown in regarding the size of the corpuscle as the only significant factor is quite unjustified.

SUMMARY AND CONCLUSIONS

The long-established theory that white corpuscles move from the axial to the peripheral stream of the blood by virtue of their lower specific gravity is reviewed in the light of the work of Fahreus and Vejlen in which this process is denied. It is pointed out that as nearly half of the volume of the blood consists of red blood corpuscles, and as these corpuscles move with differing velocities according to their distances from the intima, a series of collisions must ensue when erythro-

17. Hueper, W. C.: (a) *Am. J. Path.* **18**:895, 1942; (b) **21**:1021, 1945; (c) *Arch. Path.* **39**:117, 1945; (d) **41**:130, 1946.

18. Katz, L. N., and Dauber, D. V.: *J. Mt. Sinai Hosp.* **12**:382, 1945.

cytes or erythrocyte clumps overtake leukocytes. The result of these collisions is to force the leukocytes into the peripheral stream. The mass of the corpuscle, which is the product of its volume and specific gravity, and the relative velocities of the corpuscles concerned, are the main relevant factors that decide to what extent this "bouncing out" will occur. Evidence is brought forward to the effect that the lipophage will have a specific gravity less than that of other leukocytes, and perhaps even less than that of plasma. The velocities are considerably reduced in the large elastic arteries during diastole, and in the slow peripheral zone and adjacent portions of the axial stream gravity may also have some influence in bringing these lipophages into contact with the intima, facilitating their entrance therein and so producing the earliest lesion of atheroma.

INFLUENCE OF LOCAL ACIDIFICATION OF TISSUE BORDERING CANCEROUS GROWTHS

With Special Reference to the Eosinophil, the Paneth
Cell and the Acidophilic Plasma Cell

CHARLES E. BLACK, M.D.

AND

R. SORENSEN OGLE, B.S.

LANSING, MICH.

THIS paper is an outgrowth of a protracted search to determine, in the first place, why cancer of the small intestine is so uncommon; in the second place, why cancer when it does arise in the small intestine tends to grow so slowly and metastasize so late, and, in the third place, why intramural extensions of carcinoma of the small intestine are so seldom found. The low incidence of carcinoma of the small intestine, in striking contrast to the abrupt increase in that of carcinoma of the stomach above and that of carcinoma of the large intestine below, has provoked the interest of pathologists for many years. The infrequency of carcinoma of the small intestine has been attributed to various factors, such as the absence of anatomic constrictions like the pyloric and ileocecal valves, the absence of abrupt changes in structural continuity and the minimal degree of chronic irritation in this portion of the intestine. Still, none of these explanations seems wholly adequate to account for the low incidence and peculiar growth of carcinoma of the small intestine.

It is significant that for nearly a century pathologists have noted peculiar collections of acidophilic cells located in the immediate vicinity of cancerous growths, particularly in connection with carcinoma of the gastrointestinal tract. These acidophilic cells include the eosinophilic granulocyte and the plasma cell, both of which are normally numerous in the lamina propria of the small intestine, and the Paneth cell, present in the bottoms of the crypts of Lieberkühn of the small intestine. Although knowledge of the function of these three groups of cells is appallingly meager, the fact that they are occasionally seen arranged about cancerous growths, oftentimes forming a virtual barrier against the advancing tumor, is significant enough to merit considerable investigation.

From the Department of Pathology, Edward W. Sparrow Hospital.

EOSINOPHILS

Eosinophils are observed in many conditions, such as acute and subacute inflammation, allergic states, parasitic infestation and cancer, but there is no satisfactory explanation for this empiric observation. Eosinophils appear most conspicuous in inflammatory zones at a time when the host appears to be successfully combating the invader. For this reason, the local appearance of eosinophils is regarded as a favorable prognostic sign. One does not encounter local eosinophilia in every cancer examined or in every region of the same neoplasm. Eosinophils are observed most commonly about cancer in areas where the host appears to be waging a successful battle against invasion, as evidenced by disintegration and fragmentation of carcinoma cells associated with early local defensive fibrosis. Generally one does not see eosinophilia about old, inactive, scirrhous carcinoma. In certain carcinomas, particularly those of the gastrointestinal tract, eosinophils are exceptionally common. Biggart¹ observed local eosinophilia about every neoplasm of the gastrointestinal tract that he examined, regardless of the presence or the absence of secondary inflammatory changes.

Although the eosinophil is a conspicuous cell of the blood, the bone marrow and some connective tissues, its origin remains obscure. In 1880 Ehrlich² proposed the theory that there is a single and distinct strain of eosinophils, formed in the bone marrow, which are disseminated to all parts of the body by the circulating blood. Weidenrich³ maintained that there is no true strain of eosinophilic cells, believing that the cytoplasmic granules are phagocytosed particles originating from hemoglobin. Rous⁴ regarded the typical granules as protein absorbed from the intestine. Brown⁵ considered these granules as toxic substances absorbed by neutrophils. A third opinion, supported by Downey,⁶ distinguishes two types of eosinophilic cells. He considered the circulating eosinophils as developing from myelocytes and the tissue eosinophils as of local origin.

The theory of Ehrlich has gained considerable support. In 1932 Biggart¹ concluded from his studies of the eosinophils of normal and pathologic tissue that there is no distinguishing feature between those of the fixed tissues and those of the circulating blood. The mature eosinophil, with its bilobed nucleus and coarse, highly refractive granules, is common to both locations. The cytoplasmic granules give identical oxidase and peroxidase reactions, stain electively with

1. Biggart, J.: *J. Path. & Bact.* **35**:599, 1932.

2. Ehrlich, P.: *Ztschr. f. klin. Med.* **1**:553, 1880.

3. Weidenrich, F.: *Anat. Rec.* **4**:317, 1910.

4. Rous, F. P.: *J. Exper. Med.* **10**:537, 1908.

5. Brown, T.: *Bull. Johns Hopkins Hosp.* **73**:79, 1897.

6. Downey, H.: *Folia haemat.* **19**:148, 1915.

acid dyes and contain ionizable iron. Through vital staining the eosinophils are found to be actively motile, propelled by means of cytoplasmic pseudopodia. According to Jacobsthal,⁷ the eosinophils are capable of extruding their granules.

Eosinophils are frequently seen along the margins of neoplasms and in the connective tissue stroma of carcinoma. Figure 1a shows the local eosinophilia encountered at the spearhead of an advancing carcinoma of the rectum. The carcinoma cells in this region show disintegration and fragmentation, associated with early local connective tissue fibrosis. This area of eosinophilia is literally sprinkled with extracellular small uniform round eosinophilic granules, comparable to the cytoplasmic granules of the mature eosinophil. Moreover, this area stained more intensely with eosin than some of the nearby areas where the deeply basophilic carcinoma was obviously showing rapid growth. No recognizable carcinoma cells were seen in the immediate area of the eosinophilia.

PANETH CELLS

The acidophilic Paneth cells were first described by Schwalbe⁸ in 1872. Paneth⁹ in 1888 brought these cells to the attention of investigators, and they are now recognized as constant constituents of the glands of the small intestine. The incidence of the Paneth cells is greatest in the ileum and the jejunum, where they are about equal in distribution, while they occur less frequently in the duodenum and the appendix. It is estimated that there are about twenty Paneth cells in each crypt, and there are estimated to be four to five million crypts, which would make an estimated total of eighty to one hundred million Paneth cells. Paneth cells may occur occasionally in the large intestine and stomach, particularly under certain pathologic conditions. Their common location is in the fundi of the crypts of Lieberkühn of the small intestine. Paneth cells contain many uniform coarse cytoplasmic granules, which stain deeply with eosin and other acid dyes. These cells contain a spherical nucleus, which is located near the base, is poor in chromatin and usually reveals a nucleolus. The granules are located between the nucleus and the lumen of the gland of Lieberkühn.

Paneth⁹ regarded these cells as a specific kind of gland cell, wholly different from the goblet cell. Klein¹⁰ in 1906 found that the cells responded to physiologic stimulation, a finding which led him to consider that they represent a zymogenic cell involved in digestion. By differential staining, Dunn and Kessel¹¹ indicated that the Paneth

7. Jacobsthal, E.: *Virchows Arch. f. path. Anat.* **234**:12, 1921.

8. Schwalbe, G.: *Arch. f. mikr. Anat.* **8**:92, 1872.

9. Paneth, J.: *Arch. f. mikr. Anat.* **31**:113, 1888.

10. Klein, S.: *Am. J. Anat.* **5**:315, 1906.

11. Dunn, T., and Kessel, A.: *J. Nat. Cancer Inst.* **6**:113, 1945.

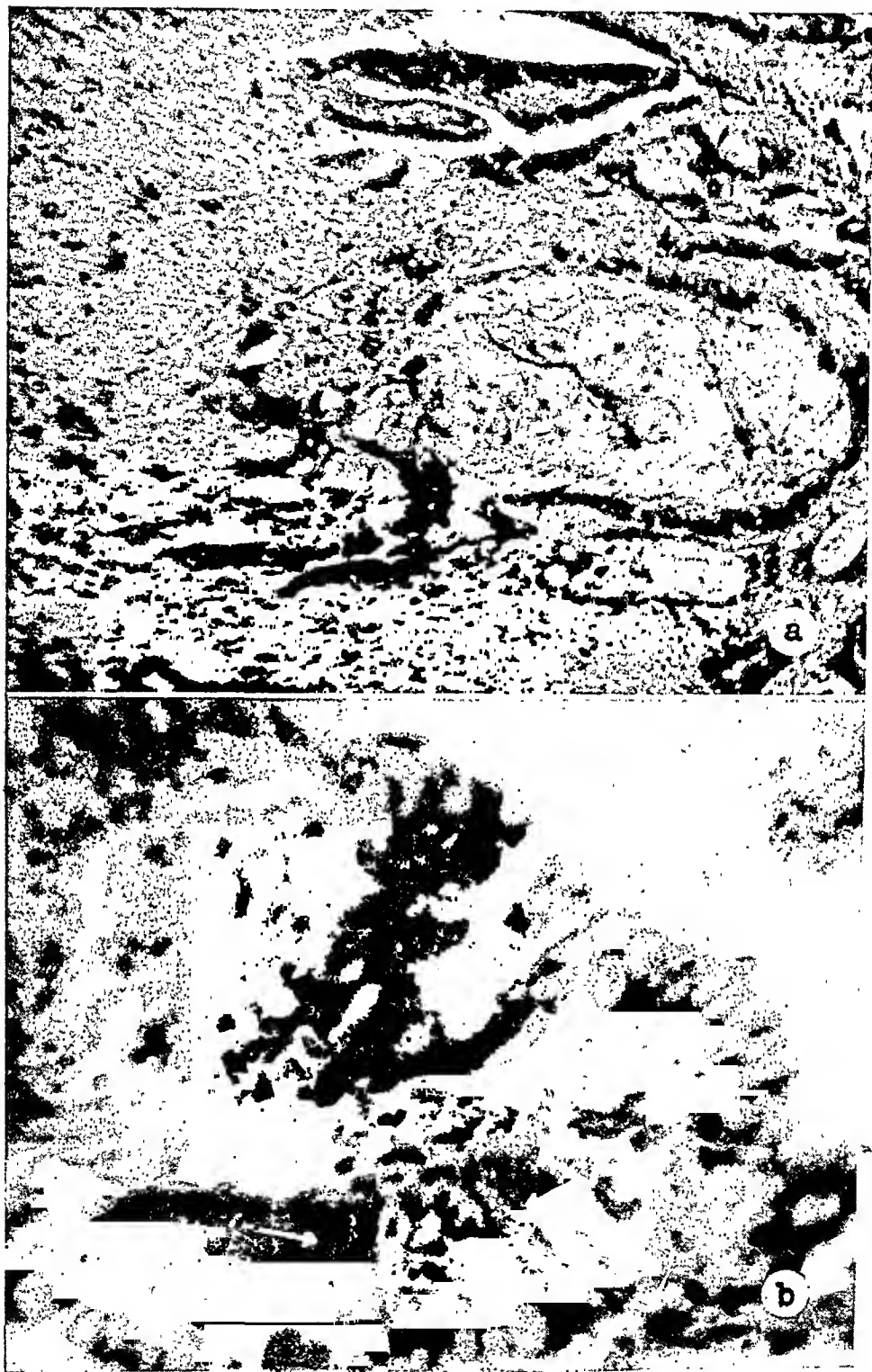


Fig. 1.—An advanced adenocarcinoma of the ampulla of the rectum in a white man aged 56: (a) The spearhead of the advancing carcinoma is shown where marked local eosinophilia was encountered, accompanied by dispersed extracellular eosinophilic granules. The foremost carcinoma cells show fragmentation and disintegration; eosin-methylene blue; $\times 1,872.5$. (b) Paneth cell hyperplasia occurring in the adenocarcinoma; eosin-methylene blue; $\times 1,872.5$.

cell granules contain a zymogenic substance. These granules accumulate during starvation and disappear during digestion. As shown in figure 2, these granules are occasionally found in the lumens of the crypts of Lieberkühn, and the cells can therefore be interpreted as having a secretory function in digestion. Herzog¹² observed that when Paneth cells exist in the stomach or the large intestine in association with carcinoma the local area is more eosinophilic, indicating local acidification of the tissue.

The acidophilic Paneth cell and carcinoma have an interesting relationship in regard to incidence. Where Paneth cells are numerous, as in the small intestine and the appendix, carcinoma is uncommon. We have found Paneth cells to be normally numerous in the unaltered mucosa of the appendix, as shown in figure 2.

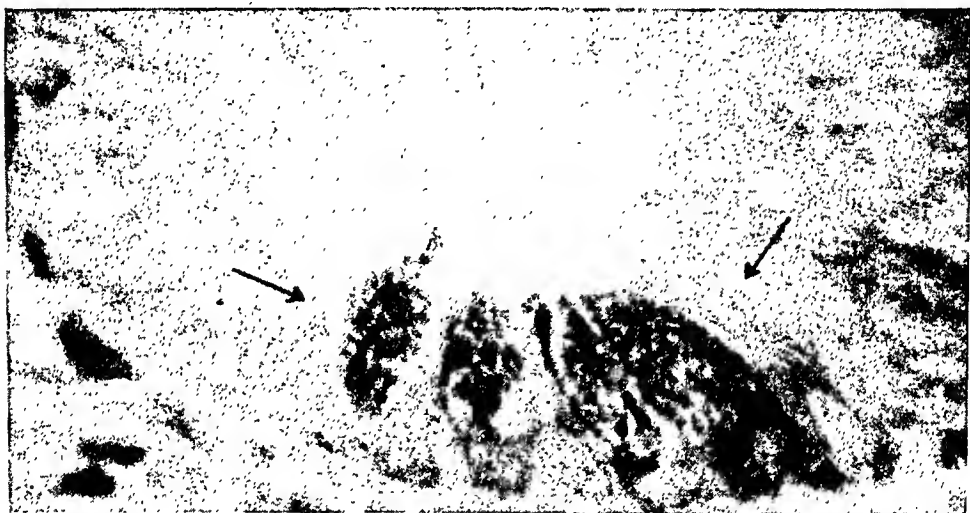


Fig. 2.—Appendix vermiformis of a white man, showing Paneth cells with some of their granules extruded into the lumen of the gland; eosin-methylene blue; $\times 1,872.5$.

Abnormal Paneth cell proliferation is encountered in connection with carcinoma of the stomach and with carcinoma of the large intestine. Paneth cells are seldom found normally in either of these locations. In figure 1 *b* hyperplasia of Paneth cells is seen in connection with carcinoma of the rectum. Paneth cell proliferation in Brunner's gland is frequently observed in cases of carcinoma of the stomach. Normally only an occasional Paneth cell is found in Brunner's glands of the duodenum.

ACIDOPHILIC PLASMA CELLS AND RUSSELL BODIES

Near the turn of the century the large globoid acidophilic bodies (Russell bodies) often seen in the region of certain cancers attracted

12. Herzog, A.: *Am. J. Path.* 13:351, 1937.

* much interest and provoked much discussion. These peculiar round acidophilic bodies were noted as early as 1858 by Fox.¹³ In 1890 Russell¹⁴ fully described them. He recognized them as "cancer parasites" of blastomycetic nature. In 1901 Hektoen and Reisman¹⁵ accurately defined Russell bodies as "peculiar, globular, homogeneous, extra-cellular and intra-cellular formations of varying size; frequently they are aggregated in mulberry-shaped conglomerations. They stain red with acid fuchsin and deep blue with the Gram-Weigert stain. These bodies occur in many normal tissues and in a great variety of pathologic processes, a favorite place for their study being glandular proliferations of the mucous membrane of the stomach (Hansemann, Thorel)."

According to Schridde¹⁶ the majority of plasma cells contain Altmann granules, while a smaller number possess fine bluish gentianophilic granules, the precursors of Russell bodies. These gentianophilic granules, through growth and confluence, give rise to the larger and more characteristic Russell bodies. With Pappenheim's differential staining method employing safranin and methyl green, Russell bodies stain red in a background of green. These bodies have a strong affinity for the acid dyes, staining red with acid fuchsin or eosin. Their staining reactions indicate that they are homogeneous and semifluid in character, being composed of a hydrophilic acid-like protein substance.

There has been much controversy over the significance of Russell bodies. Most workers regard them as degenerative products of the plasma cell, while some workers believe they are secretory in nature. Downey¹⁷ in 1911 and Kingsley¹⁸ in 1924 presented the view that the Russell body is probably a normal secretion of the plasma cell. In 1931 Michels¹⁹ presented a comprehensive review of the morphologic aspects, the function and the development of the plasma cell, including Russell bodies. His summary of the theories concerning the function of the plasma cell is as follows: (1) absorption of necrotic chromatin material; (2) metabolism of nuclear material; (3) physiologic secretion; (4) production of antibodies; (5) phagocytosis. There is evidence that the acidophilic plasma cell and the associated Russell bodies, in particular, have a secretory function. In certain situations these acidophilic cells appear to be playing a role of defense against the invader.

13. Fox, W.: *Med.-Chir. Tr.*, London **41**:361, 1858.

14. Russell, W.: *Brit. M. J.* **11**:1356, 1890.

15. Hektoen, L., and Reisman, D.: *American Textbook of Pathology*, Philadelphia, W. B. Saunders Company, 1901, p. 93.

16. Schridde, H.: *Pathologische Anatomie*, Jena, Gustav Fischer, 1921.

17. Downey, H.: *Folia haemat. (Teil 1)* **11**:275, 1911.

18. Kingsley, D.: *Anat. Rec.* **29**:1, 1924.

19. Michels, N.: *Arch. Path.* **11**:775, 1931.

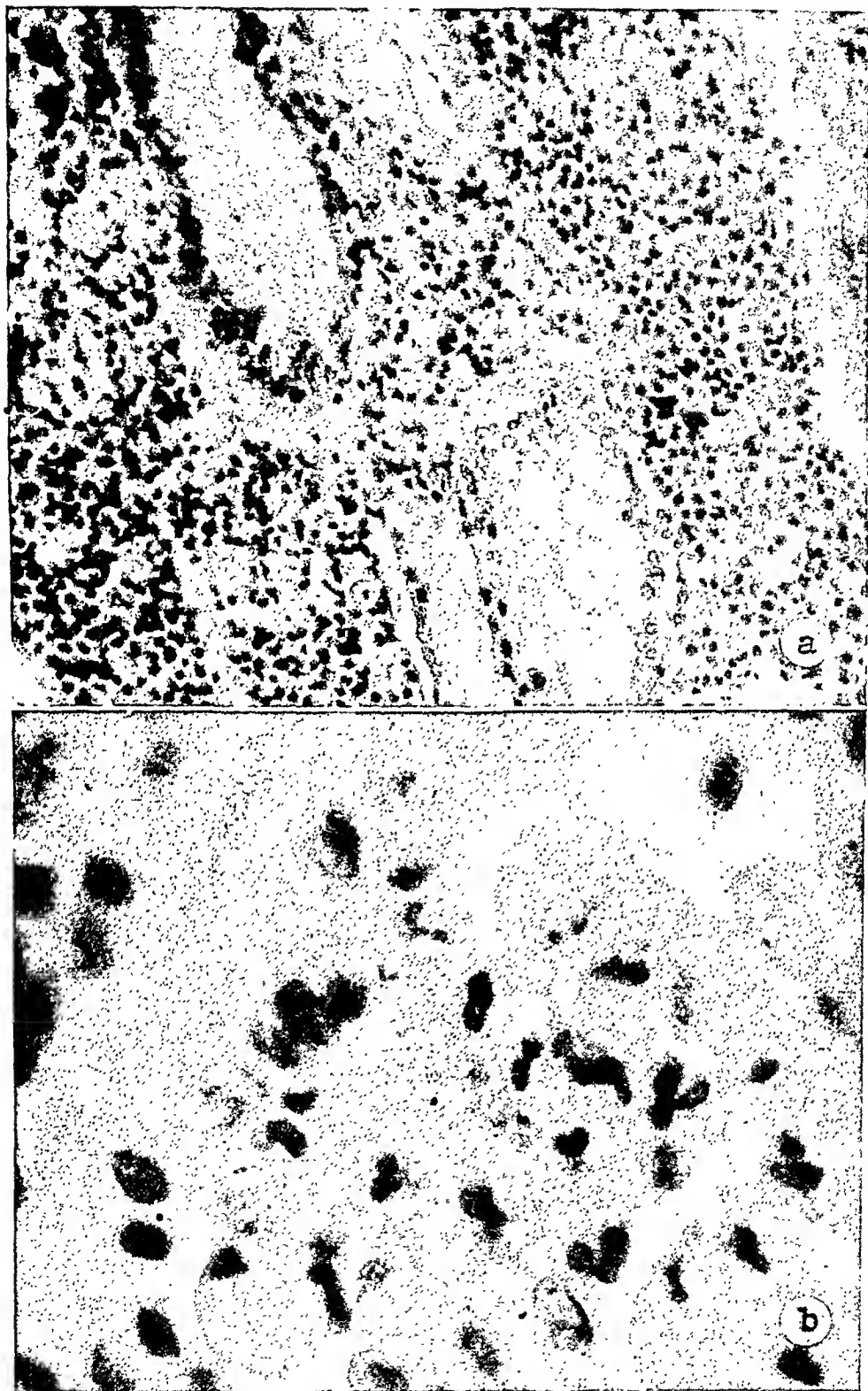


Fig. 3.—A highly invasive, poorly differentiated cell adenocarcinoma of the pylorus occurring in a white woman aged 22: (a) junction of the pylorus and the duodenum at the pyloric fold, showing acidophilic plasma cells and Russell bodies surrounding the duodenal glands adjacent to the carcinoma; hematoxylin and eosin; $\times 350$. (b) The same field under higher magnification; hematoxylin and eosin; $\times 1,900$.

The acidophilic plasma cell reaction with Russell body formation found about neoplasms, as described by many early workers, such as Lubarsch,²⁰ Saltykow,²¹ Thorel,²² Schridde²³ and Fabian,²⁴ is shown in figure 3 *a* and *b*. In the case illustrated, pyloric carcinoma of the stomach is sharply delineated at the junction of the duodenum. Great numbers of large acidophilic plasma cells and Russell bodies are seen densely packed about the duodenal glands, immediately adjacent to this neoplasm. No carcinoma cells are found on the duodenal side among the acidophilic plasma cells. An area under higher magnification in the same field next to the carcinoma on the duodenal side, demonstrating large acidophilic plasma cells and eosinophils bordering the neoplasm, is shown in figure 3 *b*.

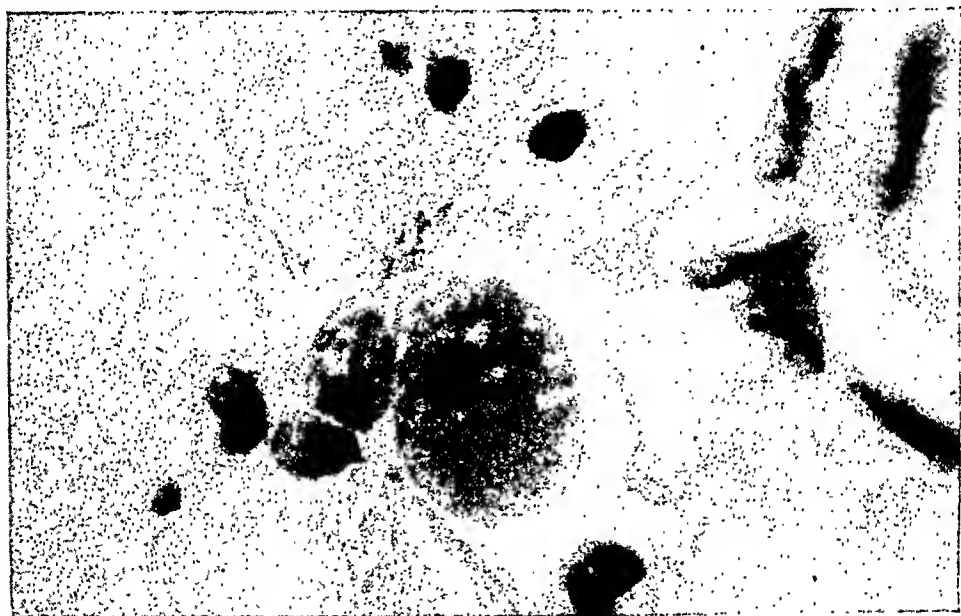


Fig. 4.—An advanced, ulcerating adenocarcinoma of the sigmoid colon densely adherent to a loop of the ileum of the small intestine occurring in a white woman aged 60. A group of acidophilic plasma cells are seen in the wall of the ileum located in the immediate vicinity of the carcinomatous attachment; hematoxylin and eosin; $\times 1,872.5$.

Secondary carcinoma seldom invades the wall of the small intestine, even when the neoplasm is attached to the serosal surface. The photomicrograph shown in figure 4 was taken from a section through the wall of the ileum where an advanced, ulcerating adenocarcinoma of the large intestine was densely adherent to it. Large numbers of acido-

20. Lubarsch, O.: *Ergebn. d. allg. Path. u. path. Anat.* **12**:180, 1895.

21. Saltykow, S.: *Virchows Arch. f. path. Anat.* **153**:207, 1898.

22. Thorel, C.: *Virchows Arch. f. path. Anat.* **151**:319, 1898.

23. Schridde, H.: *Arch. f. Dermat. u. Syph.* **73**:107, 1905.

24. Fabian, E.: *Centralbl. f. allg. Path. u. path. Anat.* **18**:689, 1907.

philic plasma cells and Russell bodies were found in the wall of the small intestine immediately adjacent to the carcinomatous attachment. A group of these large acidophilic plasma cells is shown in figure 4. Although this carcinoma was widely invading the wall of the sigmoid colon, it had scarcely penetrated the wall of the adherent loop of the ileum.

COMMENT

Observations have been presented showing that an acidophilic inflammatory cell response is made to invading carcinoma of the gastrointestinal tract. Many workers believe that the inflammation associated with neoplastic processes may represent resistance of the host. Ewing²⁵ emphasized that an inflammatory reaction frequently meets the invasion of tumor cells. He regarded it as a highly significant feature of cancerous conditions and stated that it must be regarded as a defensive process. Goforth and Snoke²⁶ expressed the belief that the many eosinophils accumulated locally about cancerous growths constitute body resistance. They concluded that the eosinophils present about carcinoma of the uterine cervix are of good omen. Schoch,²⁷ in studying a large group of cases of carcinoma of the cervix, found local eosinophilia in 40 per cent of the cases in which the patient survived for a five year period. Pavlovsky and Widakowich²⁸ also expressed the belief that local eosinophilia is a protective measure against cancer invasion. Gill,²⁹ in reviewing local eosinophilia in cases of cancer, concluded that abundant local eosinophilia is of good prognostic import and probably represents better than usual resistance to the advance of the neoplasm.

As previously mentioned, the function of the eosinophil is obscure. In general, an inflammatory reaction follows a well delineated pattern. As originally shown by Menkin,³⁰ in an inflamed area the local hydrogen ion concentration of the tissue is at first alkaline but becomes progressively acid as the inflammation proceeds. As the p_H changes, the type of inflammatory cell changes. Polymorphonuclear leukocytes flourish in an alkaline medium. According to Menkin,³¹ with the local rise in acidity (p_H 6.5 or below) the neutrophilic polymorphonuclear leukocytes become injured and quickly disappear, leaving macrophages, eosinophilic cells, lymphocytes and plasma cells unimpaired. It is generally recognized by students of inflammation, that local acidification of the inflamed area is one of the important tissue defense reactions

25. Ewing, J.: *Neoplastic Diseases*, ed. 4, Philadelphia, W. B. Saunders Company, 1940, p. 35.

26. Goforth, J., and Snoke, P.: *Am. J. M. Sc.* **175**:504, 1928.

27. Schoch, E.: *Zentralbl. f. Gynäk.* **50**:2895, 1926.

28. Pavlovsky, A., and Widakowich, V.: *Semana méd.* **1**:1265, 1926.

29. Gill, A.: *J. Lab. & Clin. Med.* **29**:820, 1944.

30. Menkin, V., and Warner, C.: *Am. J. Path.* **13**:25, 1937.

31. Menkin, V.: *Arch. Path.* **41**:376, 1946.

against invading bacteria and other injurious agents. It is quite conceivable that certain acidophilic cells, such as the eosinophilic cell, may play an important function in acidifying the tissue, making it untenable for such injurious agents as bacteria or cancer cells through the dispersement of eosinophilic granules.

As the repair stage of inflammation approaches, the number of polymorphonuclear neutrophils becomes reduced and that of the polymorphonuclear eosinophils, plasma cells and lymphocytes is increased. Kirk,³² in reviewing the clinical significance of eosinophilia, expressed the belief that the local appearance of eosinophils is an allergic response to sensitization of tissues which have absorbed the split proteins associated with local necrosis. It would seem easier to interpret the eosinophil as a defense cell in contrast to a scavenger cell. In the interpretation of cytoplasmic inclusions,³³ the fact that the eosinophilic cytoplasmic granules of the polymorphonuclear eosinophil are regularly round to oval, stain uniformly, are uniform in size, possess a strong affinity for the acid stains and are not associated with degenerative changes of the cell indicates that the granules are more likely secretory in nature. This belief is further supported by the fact that eosinophils do extrude their granules in particular locations. Eosinophils are not characteristically found in the immediate vicinity of suppurating inflammatory processes, but instead are usually in the background bordering the inflammatory or the neoplastic process. They are characteristically found in situations where the tissue responses appear to be winning the battle against the invader.

Many tissue stains, particularly eosin, are virtual acid-base indicators. By means of the modified eosin-methylene blue method,³⁴ with the eosin adjusted to a pH 3, the eosinophilic granules stain best, indicating that they are strongly acid. In areas where these eosinophilic granules are present the whole field stains more acid. This is also true in locations where there are large numbers of acidophilic plasma cells, Russell bodies and Paneth cells.

It is curiously interesting that carcinoma of the stomach rarely extends beyond the pyloric fold into the duodenum. It is also interesting that this is the site where large aggregates of acidophilic plasma cells, Russell bodies and eosinophils occur, appearing as defenders on the side of the duodenum, as shown in figure 3. Hyperplasia of the Paneth cells is frequently encountered in this same region in cases of carcinoma of the stomach.

The appendix, like the small intestine, is rarely the site of a primary carcinoma, in contrast to the common occurrence of carcinoma of the

32. Kirk, R.: *J. Lab. & Clin. Med.* **23**:1137, 1938.

33. Black, C.: *J. Infect. Dis.* **67**:42, 1940.

34. Stovall, W., and Black, C.: *Am. J. Clin. Path.* **10**:1, 1940.

large intestine, which is puzzling when one considers that the vermiform process is anatomically a part of the large intestine. From the standpoint of irritation the appendix is as vulnerable if not more vulnerable than the large intestine itself. The appendix is different from the large intestine inasmuch as it normally contains many eosinophils and plasma cells in the lamina propria and many Paneth cells in the crypts of Leiberkühn. It is strikingly similar to the small intestine in this respect. That these acidophilic cells occur normally and prominently in regions of the intestinal tract where primary or secondary carcinoma is uncommon seems more than a coincidence. It is quite conceivable that these cells play a role in maintaining a certain hydrogen ion concentration that makes the soil of these regions unsuitable for the development and growth of carcinoma.

It is generally presumed that the secretions of the small intestine are entirely of an alkaline nature. Robinson, Luckey and Mills³⁵ demonstrated that the small intestine throughout its length adjusts the hydrogen ion concentration of its contents to fit a definite pattern. They found by experimentation that an acid-base adjustment occurred in the contents of the small intestine from higher or lower prevailing values to those values characteristic of different portions of the intestine. McGee and Hastings³⁶ have made the suggestion that there are cells in the intestine which can secrete acid, and others which can secrete base. It is likely that the Paneth cells of the small intestine have such an acid-secretory function.

It is interesting that the acidophilic cells are seldom the source of primary neoplasms. Although Dunn and Kessel¹¹ interpreted a neoplasm occurring in a mouse as a Paneth cell carcinoma, no cases have been reported in which such a tumor was observed in human beings. It is also interesting in this connection that Foot³⁷ noted that carcinoma of the stomach composed of eosinophilic parietal cells is not encountered; most of the neoplasms arise in the chief cells of the gastric glands.

Local acid-base equilibrium and the development of cancer would seem to warrant much investigation. To transgress into the realm of speculation, the two organs of the body that manifest a constant acid-base flux, the stomach and the lungs, are the two sites of the whole body in which carcinoma most commonly develops. Moreover, the fact that achlorhydria is common enough in cases of carcinoma to be used as a diagnostic test adds further significance to the acid-base idea. The fact that achlorhydria is not always present in gastric carcinoma

35. Robinson, C.; Luckey, H., and Mills, H.: *J. Biol. Chem.* **147**:175, 1943.

36. McGee, L., and Hastings, A.: *J. Biol. Chem.* **142**:893, 1942.

37. Foot, N.: *Pathology in Surgery*, Philadelphia, J. B. Lippincott Company, 1945, p. 222.

does not necessarily discredit this possibility. It is conceivable that an acid-base disturbance could occur in a small area of the stomach without appreciably altering the free hydrochloric acid secreted into the stomach as a whole. It is quite probable that local alkalinization of the tissue for a long period, as in instances of old chronic catarrhal inflammation of the lungs, the stomach, the large intestine or the uterine cervix, could be an important predisposing factor in the development of carcinoma.

SUMMARY

Photomicrographs are presented showing eosinophilic cell, Paneth cell and acidophilic plasma cell reactions about carcinoma of the gastrointestinal tract. Local acidification of the tissue is found where these three groups of cells are increased, as indicated by the eosinophilic reaction of the tissue. Carcinoma cells are shown to be fragmenting and disintegrating in these acidophilic areas. It is concluded that local acidification of the tissue is a defensive response of the host. Further, it seems plausible that the eosinophils, the Paneth cells and the acidophilic plasma cells commonly occurring in the small intestine and the appendix could be an important factor in explaining why cancer is so uncommon and tends to grow so slowly and metastasize so late in this region of the gastrointestinal tract. Moreover, the large numbers of acidophilic cells occurring in the region of the pyloric fold may explain why carcinoma of the pylorus of the stomach seldom extends beyond the pyloric fold into the duodenum.

POSTMORTEM EXAMINATION OF TEETH AND SUPPORTING STRUCTURES TO AID IN PERSONAL IDENTIFICATION

MYRON J. VAN LEEUWEN, D.D.S.

BOSTON

AS LONG as wars, explosions, fires, murders or other kinds of major or minor catastrophes persist, the need for establishing the personal identities of death victims will be present. In this paper I shall attempt to demonstrate various specific methods and means, as well as some general considerations in the dental field, which can be used for the purposes of identification.

The recent war has demonstrated how the bodies of hundreds of men, women and children have been identified wholly or in part by their teeth and supporting structures. Ström¹ described how the remains of 211 Norwegian patriots, murdered by the Nazis, were identified. These bodies were buried in a common grave and all were destroyed beyond recognition. Since none could be identified by physical appearance, the jaws were sawed off and kept in specially labeled jars. Through the cooperation of Norwegian dentists who furnished dental records, 156 of these bodies were identified on dental evidence. The roentgenogram was valuable in this particular examination.

Another case was that of a Norwegian army plane that had been shot down in flames. The bodies of the two officers were charred beyond recognition. One of the fliers was positively identified by a metal post that was found, undamaged by the heat, in the root canal of a tooth where it had supported an anterior crown. Thus identity was established on the basis of a detailed dental record.

In another article Ström² referred to the identification of 25 victims, burned beyond recognition in a fire in Oslo, Norway, in 1938. All of the bodies were positively identified, 6 entirely on the basis of dental records. In 10 of the cases the dental examination was the most reliable evidence, with other factors aiding in the identification. In the remaining 9 cases no dental records were available, and identity was established by other means. Without the dental evidence, however, it would have been impossible to establish positive identifications of many of these victims.

From the Harvard School of Dental Medicine.

1. Ström, F.: *Norske. tannlaegeforen. tidende* 56:153, 1946A.

2. Ström, F.: *Odont. tidskr.* 54:443, 1946.

Perhaps one of the most well known cases of identification is that of a Dr. Parkman who was murdered about one hundred years ago by a Dr. Webster. Identity was established mainly by the finding, among badly charred bones and ashes of the victim's body, three artificial porcelain teeth and a few grains of melted gold. The remnants of the bones of the lower jaw were fitted together and the victim's dental surgeon was able to identify his earlier patient with certainty by means of a peculiar depression on the left side of the lower jaw and the remnants of the artificial dentures.

In each of the cases a previously made-out dental record was instrumental in establishing identity. If no previous chart of a dental examination were available, what general information might one gather from a careful examination of the teeth and the jaws of the dead person?

The question of establishing the age of the dead person is always of utmost importance. Age may be determined rather accurately by the various stages of calcification that one finds in the crowns and the roots of the teeth. Because these teeth may be both erupted and unerupted, a roentgenogram is essential in an examination of this type. Calcification of crowns of deciduous teeth begins in utero at the embryonal age of 4 to 5 months. At 6 months in utero all deciduous teeth have begun to calcify, and at birth all deciduous teeth are in various stages of development. In addition, calcification of the four first permanent molar teeth is usually just beginning. The approximate times at which the deciduous teeth erupt into the oral cavity are as follows:

Central incisors.....	7 mo.
Lateral incisors.....	9 mo.
First molar and canine.....	12-16 mo.
Second molar.....	2 yr.

After the eruption and subsequent loss of the deciduous teeth, one sees the advent of the permanent teeth. These teeth also calcify and erupt at rather definite ages, so that by examining (grossly and roentgenologically) as little as one half of a jaw of the dead person, one may procure valuable information as to his aged. Roughly, the times of eruption of the permanent molar teeth are as follows:

First molar.....	6 yr.
Second molar.....	12 yr.
Third molar.....	17-21 yr.

Intermediary age levels may also be determined if a quadrant of the jaw is available. That is, at 9 years of age one should find 12 permanent teeth in the mouth: 8 incisors, i. e., 2 in each quadrant of the jaw, and 4 molars, i. e., 1 in each quadrant of the jaw. Normally at this age the deciduous molar and canine teeth should also be present.

At 11 years of age one should find 20 permanent teeth in the mouth: 8 incisors, 8 premolars and 4 molars.

At 13 years of age one should find 28 permanent teeth in the mouth, and no deciduous teeth remaining: 8 incisors, 8 premolars, 4 canines and 8 molars.

At approximately 8 to 10 years of age, calcification begins in the crown of the third molar tooth. The root ends of this tooth should normally be finally calcified and fully formed at about 25 years of age. Any intermediary age may thus be determined by a roentgenogram of the area of the third molar tooth either before or after the eruption of the tooth.

Beyond the age of 25 years, after complete calcification of the ends of the roots of the third molar tooth, it becomes increasingly difficult to determine the age of the patient by inspecting the teeth or even by roentgen examination. A few observations may be noted, however. After the complete root length has been reached, a determination, by roentgenogram, of the relative size of the pulp chambers of the teeth is important. In a young person little secondary dentin has been deposited, and hence there is a large pulp chamber. In an old person the pulp chamber often may be practically obliterated by the secondary dentin that has been deposited over the years. This knowledge is helpful in determining age.

After a person is 30 years of age caries often begins to develop in the cementum. In a patient whose teeth are highly susceptible to caries, accompanied by a recession of gingival tissue, one finds caries attacking the root surface of the tooth, normally covered by cementum. This type of caries is rarely found in young persons, 30 to 35 years of age, but may be extensive in patients 40 to 45 years of age or older. This is not conclusive evidence, but may possibly be of value.

Another observation is the amount of attrition, or wearing, present in the teeth. This cannot be an accurate observation, because habit and occupation, among other things, could cause teeth of a young adult to be much worn and to have the appearance of teeth of an old person. During the era of the dust bowl storms, some years ago, it was not uncommon to see young adults with teeth worn perfectly flat, with the bite closed as much as 4 to 6 mm., because of the excessive amount of fine grit and sand in the air. Generally speaking, however, the cusps of the teeth of an old adult are worn flat and smooth, while the cusps of a young person are unworn and sharp with marked interdigitation.

In an edentulous person, the general appearance of the mandible is all one has that will help differentiate a young from an old person. In the old adult one finds a generalized uniform thinning of the bony structure of the mandible. Depressions may exist in the bony structure

of the old adult which at one time may have been so-called "pyorrhea" pockets. In general, the superior and the inferior diameter of the mandible would be of most use in establishing age, the greatest diameter being associated most often with the young adult and the smallest diameter with an old adult.

Disease, characterized by loss of bone, known more commonly as "pyorrhea," may be instrumental in determining the age of the person. The type of "pyorrhea" characterized by deep, individual, soft tissue pocket formation accompanied by a loss of bone is rarely a disease of the young adult. The type of "pyorrhea" characterized by a generalized loss of bone, known as diffuse alveolar atrophy, may more commonly be seen in young adults. In general, however, "pyorrhea" is an affliction peculiar to the middle-aged or older person. A dental record with "pyorrhea" pockets accurately indicated would be valuable in establishing identity because of the inability, except in rare instances, to produce new bone in these diseased areas. Thus even years later, when all teeth were lost, these areas might still show as healed depressions in the edentulous mandible or maxilla.

To determine sex from the teeth alone is practically impossible. There is such a wide variance in the size and the shape of the teeth and supporting structures within each sex that their use to determine sex is nearly hopeless. Habits, however, might throw some light on the subject. A rather common observation is a decided groove in a central or a lateral incisor belonging to a woman who did a great amount of sewing and bit off the thread with this tooth and its opponent. A cobbler is apt to have a worn area between two teeth where he habitually holds nails. A musician, in like manner, will have definite markings on his teeth where they contact his instrument. A definite depression of a tooth, as well as a wearing away, is present in every habitual pipe smoker. Further, a characteristic black, tarry stain or residue is present on the lingual surfaces of the teeth of persons whose pipe smoking is heavy. This can be differentiated from the common brown stain caused by cigaret smoking—found usually in the lower anterior region. Thus indirectly, through habit, one may gain some information as to the sex of the dead person.

Besides age and sex, one would like to be able to determine other characteristics which might be of importance in identifying the victim of unexplained death. If a crown of an upper central incisor is available, one may obtain some knowledge as to facial contour, height and perhaps type of build from it. By inverting the tooth and allowing the incisal edge of the crown to correspond to the hair line of the owner and then looking at the labial aspect, one can roughly determine the shape of the face. Facial shapes fall into three classifications, i. e., square, tapering and ovoid. In carrying this further, the long, thin,

square crown would indicate a long, thin, square type of face; a tapering crown would indicate a face tapering markedly in the region of the chin; an ovoid crown would indicate a round-faced person. These rules are not infallible, but the resemblance is striking enough in most cases to be more than just coincidence.

Occasionally in a dental examination a hard, bony structure is discovered in the midpalatal area of the patient, the so-called torus palatinus. This bony growth may be from 5 to 25 mm. in diameter and persists throughout life. Occasionally, in like manner, hard, bony protuberances from 3 to 10 mm. in diameter may be found unilaterally or bilaterally on the inner surface of the mandible on each side of the midline. When found here they are called torus mandibularis. These develop in the young adult and persist throughout life, remaining even after the loss of all teeth unless removed surgically. If a previous knowledge of their existence has been recorded, they may contribute to identification.

Another facial characteristic which may be valuable in establishing identity is the position of the upper relative to the lower jaw. Any close friend or even casual acquaintance retains a mental picture of a general facial appearance. In an attempt at establishing identity, even if the soft tissue has been completely lost, the jaws may be reassembled in their characteristic position. This position can easily be reestablished by matching worn surfaces where the teeth contact in normal occlusion. From this reassembled articulation a description can be written which could be recognized by a member of the family, a friend or even an acquaintance. The jaws of the victim when articulated might fit in such a manner as to cause the teeth in the lower arch to project anywhere up to 25 to 30 mm. anterior to the upper arch. This of course would be in a person with a prominent chin. If the lower arch were anywhere up to 10 to 20 mm. posterior to the upper arch one would visualize a person with a marked receding chin. The normal relationship obtains when the lower teeth bite just lingual to the upper teeth with no space between them.

Other distinguishing characteristics might be: spaced anterior teeth either in the upper or in the lower arch or in both. Often these are outstanding in a person's appearance. Teeth may be beautifully in line or there may be irregularity of one or all teeth. There may be an uneven wearing of one or all of the anterior teeth. There may be areas of hypoplastic or discolored enamel, owing to a defect in enamel formation. There may be characteristic notches, grooves or fractures which would give a person a characteristic appearance. A person living in an area where fluorine is present in the drinking water in excess of 2 to 4 parts per million during the period in which his teeth

were developing would have characteristic yellowish brown pigmented areas in the enamel. If the condition is sufficiently severe, the term "mottled enamel" is applied. This characteristic mottled appearance remains throughout the life of the person and is an excellent aid in establishing identity.

It has been found further that teeth which have developed in a high fluorine area have a much higher fluorine content than teeth which have developed in a nonfluorine area.⁸ One may assume, then, that a determination of fluorine made on the ash of the tooth might help establish identity by proving that the person either lived or did not live in a particular area during the time his permanent teeth were developing.

The part that the teeth themselves play in a positive identification cannot be overemphasized. Each tooth has five surfaces, any one of which may become carious and require dental attention in the form of a restoration or restorations. Thus a combination of restorations may be present in any given mouth at any time. In addition, various types of restorative materials are routinely used, which would further individualize a mouth so that it could be distinguished from another.

Also, inlays, crowns and fixed bridges might be present in a particular mouth, the outline, the contour and the construction of which would be peculiar to that mouth alone. Suppose that a partial denture was present. If one considers individual variations as to tooth size and shape, tissue form, bone contour and arch size and shape, it becomes apparent that a partial denture could not possibly fit any mouth but the mouth of the person for whom it had been constructed.

Granted, then, that no two human dentitions are identical, one sees the immense value of adequate records made by practicing dentists and the necessity that they be available at all times for the purposes of identification.

If dental records are not available, a careful examination of the remaining teeth may still give much information. Dental practitioners do various types of dentistry, and thus the quality of the dental work may be established. For instance, a mouth that had been entirely reconstructed with gold inlays and bridges with beautifully fitted margins would place the subject in a comparatively high income group. Well fitted inlays and poorly fitted inlays or poorly formed amalgams in the same mouth might indicate that more than one dentist had worked on that particular mouth. Poor dental work and good dental work in the same mouth might indicate a change of economic level in the lifetime of the person; certainly, a change of dentists would be indicated. These factors might be helpful in establishing identity.

3. Armstrong, W. D., and Brekhus, P. J.: *J. Dent. Research* **17**:27, 1938.

Gustafson⁴ recently demonstrated another important method of identification. He has shown that by making a careful microscopic examination of ground sections of teeth under polarized light it is possible to determine accurately which teeth of a given group of teeth came from the same person. This is done by comparing Ebner's lines in the dentin on one tooth with corresponding Ebner's lines in another tooth. If subsequent to an explosion or some other mutilating accident, only a number of teeth remained, a method such as this would prove invaluable in determining whether the teeth belonged to one or more than one person; in fact, the exact number of persons involved could be accurately determined.

In the recognition of injury, several factors may be of importance. A case is called to mind in which no marks of violence were found anywhere on the skeletal remains except in the jaws and the teeth. Careful examination of the jaws and the teeth showed that the enamel of various cusps of opposing teeth had been fractured. Besides the fractured enamel of these cusps, it was discovered that the left side of the lower jaw was fractured just below the head of the condyle. By assembling the jaws in their proper positions and by noting that buccal cusps of the upper jaw and lingual cusps of the lower jaw on the left side were fractured, it became quite apparent that a hard blow must have been delivered to the right side of the lower jaw in order for those particular cusps to be fractured in those particular places as well as for the mandible to be fractured.

In the case described there was no soft tissue to aid in the examination. When soft tissue is present, additional information may be obtained. A blow delivered to the jaw before death would in all probability loosen a tooth or teeth. This loosening would be accompanied with hemorrhage and compression of the supporting bone of the tooth socket. Blood cells present around the neck of the tooth or blood cells infiltrating into adjoining tissue spaces, or both, would indicate antemortem loosening. If extracellular blood could not be demonstrated, the presence of foreign body cells, phagocytic in type, might be demonstrated in tissue spaces where hemorrhage had been. If a tooth had been loosened post mortem, in transferring the body from one place to another, neither extracellular blood nor foreign body cells could, of course, be demonstrated.

Likewise, antemortem loss of a tooth may be differentiated from postmortem loss. If a tooth is lost ante mortem, hemorrhage occurs and a clot forms immediately (except in rare instances). A clot in the socket with organization taking place by fibroblastic proliferation would indicate healing and thus antemortem loss. Postmortem loss would show, of course, no hemorrhage, no organization and no attempt

4. Gustafson, G.: *J. Am. Dent. A.* **35**:720, 1947.

toward healing, with sharp, jagged edges of supporting bone remaining. This is entirely in opposition to nature's method of producing a smoothly rounded alveolus with resorption of sharp bony spicules following an extraction.

Differentiation of a tooth lost by extraction and a tooth lost by avulsion, or a knocking out by means of a blow, could also be demonstrated. Generally speaking, a tooth extracted by an oral surgeon or a dentist would show some evidence of surgical procedure, i. e., flap formation to facilitate removal of the tooth or root, presence of bone grindings produced by a surgical burr, filed edges of a bony socket or cleancut areas of bone produced with a rongeur to produce a uniform smooth result. Avulsion, on the other hand, in all probability would present a rather crude picture. Even if the tooth were completely knocked out of the socket, the jagged edges of remaining bone, splinters of buccal or lingual plate, areas of compressed bone or fractures of roots or crowns of adjacent remaining teeth would, in all probability, exist. It would be practically impossible to knock out completely one tooth in an arch without showing some evidence of fracture, or loosening of teeth adjacent to it. This could best be demonstrated by a roentgenogram of the area along with a careful examination.

Within limits, it is possible to estimate the time that a tooth was extracted before death. If a tooth was extracted one year before death, a roentgenogram of the area would show the socket completely filled with new bone, the soft tissue, of course, being completely healed. A six month antemortem extraction would be indicated by soft tissue that had completely healed and a roentgenogram showing the socket filled with new bone but with the outline of the location of the roots still present. The picture would be similar for a two or three month antemortem extraction, with less bone having filled in the socket. A one or two day antemortem extraction would be shown by a clot with definite organization begun. A one week antemortem extraction would be shown by a definite healing and filling of the socket with organized clot. A two or three week antemortem extraction without complications would be indicated by soft tissue that had completely healed but a socket not yet filled with bone. This would have to be demonstrated, of course, by roentgenogram.

In regard to the relative degrees to which teeth and bones may be destroyed by heat, Schirnding⁵ observed:

In the great fire of the . . . Opera-Comique . . . in Paris . . . the effects of the high temperature . . . [showed] that . . . in most cases the teeth were better preserved than other parts of the body on account of their sheltered position. The materials used in the fillings withstood the influence of the fire to various extents, according to the grade of their composition. The teeth them-

5. Schirnding, H.: *Dent. Cosmos* 76:853, 1934.

selves were burned in the most varied ways. The most damaged were always found in skulls that had been more or less completely destroyed by the flames. In such cases the teeth were reduced to small stumps, whitened by the fire, were easily loosened from their alveoli, or had already fallen out. The charred cementum, with remains of calcinated enamel sticking to it, was also found with the root still sticking in the alveolus.

Schirnding stated further:

The changes to which the teeth are subject through the influences of high temperature are of great importance in forensic medicine. The darker coloring of those teeth [meaning those teeth subjected to great heat] that have previously been the most healthy may easily be mistaken for caries or injuries caused by some occupation, while, on the other hand, teeth that had previously been the darker ones may become lighter or even white in the process of calcination. This is especially observable in teeth that have been heavily coated with tartar. Of course, these alterations considerably increase the difficulty of making a diagnosis. In the same way, where pieces of the teeth have been broken off during or after their exposure to the influence of great heat, bodily injuries may be supposed to have been the cause, and the difficulty of identifying a person is thus increased.

The importance of establishing personal identity is evident. Continued effort on the part of members of the dental profession to record and to make available accurate examinations of the mouths of patients plus close cooperation between the dentist and the medical examiner would do much to aid in the establishing of identity by means of the teeth and their supporting structures.

CONTROL OF HEPATIC COCCIDIOSIS OF RABBITS WITH SUCCINYLSULFATHIAZOLE U. S. P.

A Study of the Mode of Action of the Sulfonamides

MICHELE GERUNDO, M.D., Ph.D.
HONOLULU, TERRITORY OF HAWAII

THERE have been few investigations concerning sulfonamide prophylaxis or therapy of protozoan diseases. The protozoa probably pursue their first life cycle in the intestinal lumen; from there the sporozoites pass to the liver through the radicles of the vena portae and reach Kiernan's spaces, whence they make their way into the bile ducts.

It was within the scope of the present investigation to ascertain whether succinylsulfathiazole U. S. P. ("sulfasuxidine" N. N. R.) can prevent the coccidia from reaching the liver or from proliferating within the intestinal lumen. Since sulfonamides may inhibit cell division or motility, it was surmised that sporulation of the parasites and invasion of new biliary duct cells might be prevented or delayed by the administration of the drug.

EXPERIMENTAL PROCEDURE

As a preliminary step, the rabbits were given succinylsulfathiazole to eliminate, if possible, any intestinal coccidia which they might harbor. The rabbits were then divided into three groups of 7 each.

Group 1. The animals received no treatment.

Group 2. Each of the animals received one single dose of 10,000 oocysts by mouth.

Group 3. Each of the animals received one single dose of 10,000 oocysts by mouth and a daily dose of 0.5 Gm. of succinylsulfathiazole mixed with the feed.

The animals were killed at the eighteenth day following infection and their organs examined. The first group, as was to be expected, showed no lesions of any sort. The animals of the second group had enlarged livers, studded with numerous small grayish nodules all through the parenchyma, and a biliary tree uniformly dilated.

The animals of the third group showed a smooth liver with no lesions of any kind visible to the naked eye. The appearance of the organ was similar to that in the first group with the exception of moderate engorgement and a slight increase in size. As the findings were identical in all the animals of the succinylsulfathiazole series, it was assumed that the sulfonamide had prevented the infection.

The oocysts which were collected aseptically from the gallbladders of the infected animals were immersed in four different solutions: a potassium dichromate solution, an aqueous solution of sulfathiazole (1:2,000), an aqueous solution of succinylsulfathiazole (1:700) and an 0.85 per cent sodium chloride solution.

Neither sulfathiazole nor succinylsulfathiazole in the concentrations noted here prevented the sporulation of the oocysts.

PATHOLOGIC OBSERVATIONS

The histologic study of the livers was made by means of paraffin sections stained with hematoxylin and eosin.

Group 1.—The liver showed normal structure with the hepatic trabeculae symmetrically arranged around the centrolobular vein and the sinusoids lined by flat endothelial cells. The spaces of Kiernan showed small slitlike biliary lumens lined by cylindric epithelial cells in one single row and a normal framework of fibroblasts and collagen fibers. The liver cells occasionally appeared granular with small vacuoles outside the cytoplasm; however, no pigment granules or other inclusions were present.

Group 2.—Under low power magnification were seen enormously dilated biliary ducts with their profusely proliferating epithelium thrown into countless papillary folds. Either free in the lumen or parasitizing epithelial cells, numerous gametocytes of *Eimeria stiedae* were seen, and occasionally also schizonts in process of division or free sporozoites. The cellular reaction around the ducts was moderate and included chiefly macrophages, plasma cells and fibroblasts; it sometimes extended to the neighboring areas of the liver, where it penetrated into the hepatic lobules. Sometimes inside the hepatic lobules islet-like areas were seen, in which newly formed biliary capillaries seemed to originate from the liver cells themselves. The liver cells were well preserved, and, except for the infiltration which distorted the normal structure, showed no signs of pathologic disturbance.

Group 3.—The structure of the liver was not greatly distorted; the Kiernan spaces were of a normal appearance except for a marked cellular reaction, which sometimes involved also the peripheral area of whole lobules. The biliary ducts were small and slitlike or round, and were lined by single rows of cylindric epithelial cells. Around the ducts and occasionally even between the cells lining the lumens were numerous macrophages, lymphocytes and fibroblasts. No parasites were found even where the cellular reaction was most pronounced. The liver cells were swollen and showed granular, vacuolated cytoplasm; their nuclei, however, were preserved and appeared normal. As the cells of untreated infected animals did not show any similar lesion, it is probable that the cytoplasmic changes were due to the administration of succinylsulfathiazole rather than to any toxic products of the parasites.

COMMENT

Most of the published results of experiments with sulfonamides carried out on protozoa deal with malaria of birds and monkeys because of the close connections with human malaria¹ in certain aspects.

1. Coggeshall, L. T.: Proc. Soc. Exper. Biol. & Med. **38**:768, 1938; J. Bact. **39**:30, 1940; J. Exper. Med. **71**:13, 1940.

Succinylsulfathiazole has not been used experimentally to date on coccidiosis; thus, so far as my knowledge extends, this is the first report of successful prophylaxis of coccidiosis of the liver by administration of this drug. The danger of hepatic damage seems slight. Lindelof² stated that sulfonamides cause an increase of tonus and an "acceleration of pendulum" of the living intestine; Climenko, McChesney and Messer,³ who studied the effects of continued administration of sulfathiazole in dogs, found that it may influence renal excretion but that it does not impair hepatic function.

How does succinylsulfathiazole act on the parasite? Chodat and Olivet⁴ found that sulfanilamide interfered with the sporulating activity of algae, and Lwoff, Nitti, Trefouel and Hamon⁵ noted that cell division of the flagellate *Polytomelia caeca* is inhibited by this same compound. Thomas was the first to observe that sulfanilamide inhibits division of eggs of the sea urchin, and Fisher, Henry and Low⁶ compared its action to that of typical narcotics. While further work on the action of succinylsulfathiazole on coccidia is being carried out in the present investigation, there is reason to believe that perhaps the mechanism of cell division or of sporulation and excystation of the parasites is impaired by the sulfonamide.

The cellular reaction encountered in the livers of animals treated with succinylsulfathiazole might be interpreted superficially as due to stimulation of immunizing mechanisms by the drug. However, the effect reasonably could represent a natural reaction of the organism to an infection of decreased intensity and thus could be indirectly related to the action of the drug itself.

This reaction which has been observed indicates that the sporozoite must have reached the liver, where either their motility was impaired or their growth and division were inhibited. As the biliary epithelium showed no deviation from the normal and no attempt at proliferation, it must be assumed that progress from the portal vein to the biliary duct had been impeded and that a cellular reaction had taken place to eliminate the parasites. Both mechanisms may be postulated for the drug action: inhibition of motility and inhibition of cell divisions, each leading to the elimination of the parasites and to the establishment of a strong cellular reaction.

2. Lindelof, S. A.: Chem. Abstr. **37**:4466, 1943.

3. Climenko, D. R.; McChesney, G. W., and Messer, F.: Proc. Soc. Exper. Biol. & Med. **46**:124, 1941.

4. Chodat, F., and Olivet, R.: Arch. sc. phys. nat. **22** (supp.):143, 1940; cited by Henry, R. J.: The Mode of Action of Sulfonamides, New York, Josiah J. Macy Jr., 1944.

5. Lwoff, A.; Nitti, F.; Trefouel, J., and Hamon, V.: Ann. Inst. Pasteur **67**:9, 1941.

6. Fisher, K. C.; Henry, R. J., and Low, E.: J. Gen. Physiol. **27**:469, 1944.

Some authors ⁷ have advanced the idea that sulfonamides may constitute a factor in the enhancement of the mechanism of immunity. As toxins are not affected by contact with sulfonamides, the increase of resistance seems only an incidental factor in the action of the drugs. The principal action is exercised on the micro-organisms themselves.

Sometimes a certain result may be due to a composite of inhibitive effects without the inhibitors having any mutual interrelationship. The simultaneous administration of a sulfonamide drug and serum, for example, may result in a more prompt recovery than administering of either one by itself, but this synergistic effect does not mean that the action of one has served to enhance the effect of the other. We can assume, instead, an independent action of each on the bacteria and a cumulative effect in stimulating or reawakening the defensive processes of the organism. Further speculation on this subject needs additional investigation.

SUMMARY

Succinylsulfathiazole has been tested on rabbits infected with *Eimeria stiedae*. In the first series of experiments, the rabbits were infected with 10,000 oocysts of *E. stiedae*, and one half of them were given 0.5 Gm. of the sulfonamide, mixed with the feed, daily for a period of 14 to 16 days. The untreated infected animals showed typical lesions of coccidiosis of the liver, whereas the infected animals treated with succinylsulfathiazole showed no lesions of the liver. Since the results were uniform in all the animals and no toxic effects were observed as a consequence of the administration of the drug, it is hoped that this widespread infection may be successfully treated. From the study of the histologic material it seems likely that the sporozoites are carried through the portal vein to the liver, where either they are inhibited in their progression toward the biliary epithelium or, if they reach there, they are prevented from dividing.

Department of Laboratories, St. Francis Hospital, Honolulu, Territory of Hawaii.

7. Findlay, G. M.: *Lancet* 2:761, 1941. Lyons, C., and Mangiaracine, A.: *Ann. Surg.* 108:813, 1938.

SIGNIFICANCE OF AGONAL CHANGES IN THE HUMAN LIVER

HANS POPPER, M.D., Ph.D

CHICAGO

DESPITE great efforts of clinicians and pathologists, the correlation of morphologic changes of the liver and clinical manifestations remains problematic. In many instances the functional significance of alterations seen under the microscope cannot be evaluated. This difficulty is met in the experimental animal when marked histologic changes are found in the absence of jaundice or definite impairment of hepatic function. In regard to the human subject the problem is augmented if the histologic studies are based on autopsy material. The occurrence of marked premortal, agonal and postmortal changes of the liver is brought home by the study of specimens obtained for biopsy; the use of such specimens has been rather extensive in recent years.¹ This problem invites a systematic investigation of the hepatic changes that occur in the agonal period and an evaluation of their significance.

To study this problem, three different approaches were used: 1. The histologic picture as seen in autopsy material was compared with that seen in biopsy specimens without any attempt to study the same liver in premortal and postmortem material. 2. In the rare cases in which a liver specimen could be obtained at autopsy a short time after a biopsy had been made, premortal and postmortal histologic appearances were compared. 3. Livers of healthy persons who died instantaneously, as in crashes, were compared with those of persons who died suddenly but with an interval of more than 10 minutes between the onset of the injury or the disease and the actual death. In cases of the latter type, therefore, an agonal period of more than 10 minutes has to be assumed.

From the Hektoen Institute for Medical Research and the Departments of Pathology of Cook County Hospital and Northwestern University Medical School.

This study was supported by a grant from the Committee on Scientific Research of the American Medical Association and a grant from the Dr. Jerome D. Solomon Memorial Research Foundation, Chicago.

1. (a) Iversen, P., and Roholm, K.: *Acta med. Scandinav.* **102**:119, 1939. (b) Dible, J. H.; McMichael, J., and Sherlock, S. P. V.: *Lancet* **2**:402, 1943. (c) Mallory, T. B.: *J. A. M. A.* **134**:655, 1947. (d) Jones, M. C., and Volwiler, W.: *M. Clin. North America* **31**:1059, 1947. (e) Popper, H.; Steigmann, F.; Meyer, K. A.; Kozoll, D. D., and Franklin, M.: *Am. J. Med.*, to be published.

There are in the literature ample references to postmortal autolytic processes, which are especially rapid in cases of so-called acute atrophy of the liver. This was pointed out by Umber,² Versé³ and Hanser⁴ many years ago, after comparing a biopsy specimen taken during a surgical operation with autopsy material obtained shortly thereafter. In their studies emphasis was placed on the changes of the cells of the hepatic parenchyma. In my own experience,⁵ central necroses, which were absent in biopsy specimens obtained shortly before death, were observed at autopsy. That the postmortal autolytic processes are enhanced in cases of infection or toxemia is well known to every pathologist. Glycogen present in liver cells as indicated by histochemical methods or the characteristic granulation of the cytoplasm has been considered as evidence of sudden death,⁶ and medicolegal significance has even been ascribed to it.⁷

MATERIAL AND METHOD

For the general comparison of autopsy and biopsy material 226 specimens obtained by needle aspiration (performed by D. D. Kozoll^{1a}) from 156 patients and 108 specimens obtained by surgical excision during laparotomy (performed by K. A. Meyer⁸) were available. The great majority of these specimens revealed definite pathologic changes; in the surgical series, however, there were some fairly normal pictures, as, for example, in specimens from patients with peptic ulcer. Some of the biopsy specimens were fixed in formaldehyde solution U. S. P. diluted 1:10, formaldehyde-Zenker or acetic acid-Zenker solution; the majority of them were hardened in Carnoy solution, which is optimal for the study of the perisinusoidal spaces.⁹ Autopsy material fixed in different types of solutions was used for comparison.

Biopsy and autopsy materials of the same patient were available in 38 cases. In only 4 instances was the interval of time between autopsy and biopsy shorter than 48 hours, and these cases were utilized for comparison.

The third part of the study was based on material observed in two histopathologic centers of the Army; it includes 351 subjects, all males of military age; instances in which the subject was over 45 years of age were excluded. These soldiers were healthy until shortly before death and died suddenly—some from causes connected with military training and the rest from diseases or accidents not directly connected with military life. Death had occurred within

2. Umber, F.: *Berl. klin. Wchnschr.* **57**:125, 1920.

3. Versé: *Berl. klin. Wchnschr.* **57**:127, 1920.

4. Hanser, R.: *Verhandl. d. deutsch. path. Gesellsch.* **18**:263, 1921.

5. Popper, H.: *Wien. klin. Wchnschr.* **49**:207, 1936.

6. Popper, H., and Wozasek, O.: *Virchows Arch. f. path. Anat.* **279**:3, 1931.

7. Meixner, K.: *Beitr. z. gerichtl. Med.* **1**:221, 1911.

8. Meyer, K. A.; Steigmann, F.; Popper, H., and Walters, W. H.: *Arch. Surg.* **47**:26, 1943.

9. Eppinger, K.; Kaunitz, H., and Popper, H.: *Die seröse Entzündung*. Berlin, Julius Springer, 1935.

24 hours after the accident or the onset of disease. Cases in which the history revealed that symptoms of illness had been noted a longer time before death were excluded. On the basis of the information available in the history, these 351 cases of sudden death were divided into two groups: 96 instances of instantaneous death, the result of a trauma, and 255 cases in which death occurred between 10 minutes and 24 hours after the onset of a disease or the time of an accident. For comparison, sections of the livers of 160 soldiers who died of a sequela of an accident or of a disease more than 24 hours after the accident or the onset of the disease were studied.

In addition to paraffin sections stained with hematoxylin and eosin, others stained with Mallory's aniline blue stain or his modification of the Masson stain and Gömöri's or Foot's reticulum fiber stain were studied. In selected instances, slides stained for glycogen with the periodic acid method of McManus¹⁰ were examined.

RESULTS

Comparison of the Premortal and Postmortal Histologic Appearances of the Liver Based on Biopsy and Autopsy Material in General.—As repeatedly emphasized, the cytoplasm of the liver cells in hematoxylin-eosin sections of biopsy specimens was lightly stained. In the absence of marked pathologic changes it revealed a uniform, fine vacuolation and granulation. These were due to the removal of cytoplasmic glycogen which occurs in the embedding process. If the biopsy specimen was taken shortly after a therapeutic intravenous injection of dextrose, the cells appeared swollen. In autopsy material the cytoplasm was usually darker except for the presence of fat vacuoles. The cells appeared similar to those of biopsy specimens only in instances of acute death or after extensive premortal dextrose therapy. In biopsy specimens the space between liver cell cords and sinusoids was, as a rule, completely obliterated. The larger and thicker reticulum fibers which were parallel to the axis of the liver cell cords were well impregnated in silver preparations and stained with aniline blue. However, the cross fibers were not visualized in aniline blue stains, since the sinusoidal wall and the Kupffer cells appeared attached to the liver cell cords. Only in the presence of edema, which was primarily observed in toxic hepatitis, in long-standing biliary obstruction (biliary hepatitis) and especially in the central portion of the lobule in congestion, was the perisinusoidal space seen. It was almost invariably visible in autopsy specimens. It varied in width, and in a number of cases, considered instances of hepatic edema, marked albuminoid debris was seen in it; the sinusoids appeared compressed in the instances in which edema was fully developed. The sinusoidal walls with the Kupffer cells were detached from the liver cells almost without exception. The perisinusoidal space was traversed by a large number of fine cross reticulum fibers, which connected in the form of an arch and occasionally interlaced the thicker fibers adherent to the perisinusoidal wall with the liver cell cords. They appeared more in number and better impregnated than those in biopsy specimens.

Dissociation of individual liver cells from the cell cords was occasionally observed in biopsy specimens. These cells appeared round; their cytoplasm was eosinophilic. A communication between the bile capillary in the center of the cords and the perisinusoidal space could hardly be visualized, despite the dissociation. Denudation of the framework in the center of the lobule with development of central necrosis was hardly ever visible, even when the cytoplasm of the liver

10. McManus, J. F. A.: *Nature*, London **158**:202, 1946.

cells in the central portion had lost its basophilia or when definite damage of liver cells could be established by morphologic or functional findings. In autopsy specimens, however, disruption of the liver cell cords with dissociation of the cells was not uncommon, even in instances in which the damage of liver cells was not severe. The dissociated cells might reveal normal basophilia of the cytoplasm or intact nuclear staining. A funnel-shaped communication between the lumen of the bile capillary and the perisinusoidal space was occasionally demonstrable. This was especially clear if the bile capillaries were dilated in the presence of jaundice.

As to the examined experimental animals, which included mice, rats, rabbits, dogs and guinea pigs, the perisinusoidal spaces were hardly ever visible even in postmortem material; the cross fibers of the reticulum network were less clearly demonstrated than in the liver of the human subject. As far as the perisinusoidal

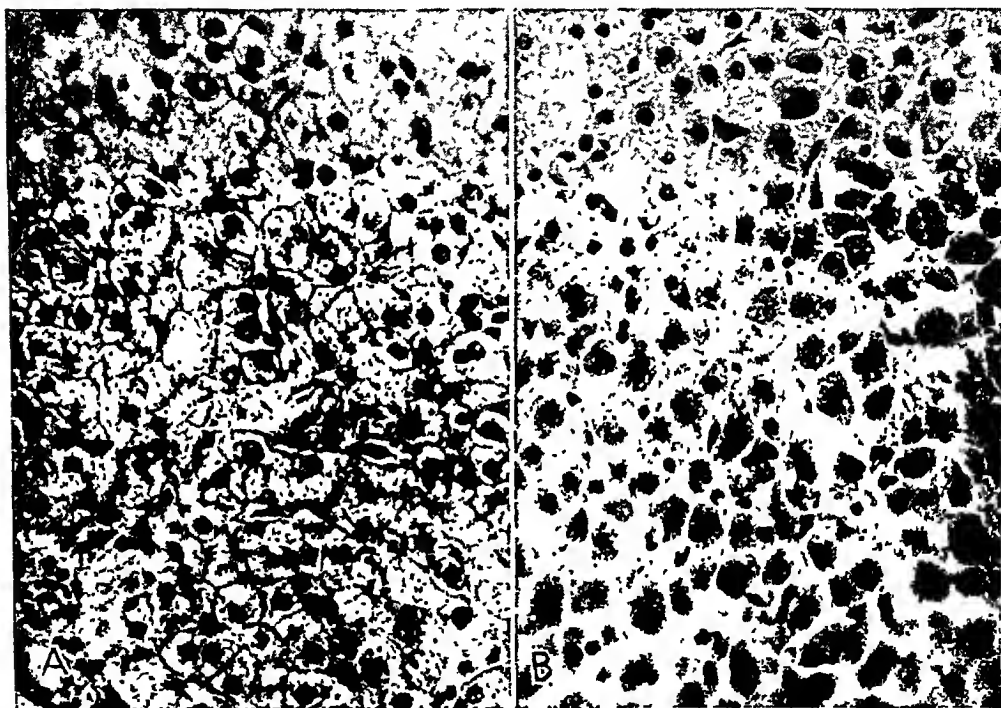


Fig. 1.—*A*, biopsy specimen of liver taken 13 hours before death in a case of mild toxic hepatitis. The structure of the liver cell cords is only slightly distorted, and the perisinusoidal spaces are almost completely closed. The liver cells appear to be rich in glycogen.

B, autopsy specimen obtained in the same case as *A*. There is marked dissociation of the liver cell cords, and the tissue spaces between them and the sinusoids are open. The isolated liver cells appear poor in glycogen.

space is concerned, the liver of the examined animals resembled human liver as seen in biopsy specimens.

Comparison of the Premortal and Postmortal Histologic Appearances of the Liver Based on Biopsy and Autopsy Specimens of the Same Liver.—Differences between biopsy and autopsy specimens the same as those described in the foregoing section of this paper could be demonstrated in the 4 cases available for this part of the study. Of special value is a case of a 66 year old Negro woman with a mild degree of toxic hepatitis without established exposure in the history. Only slight jaundice was present; the liver appeared enlarged and

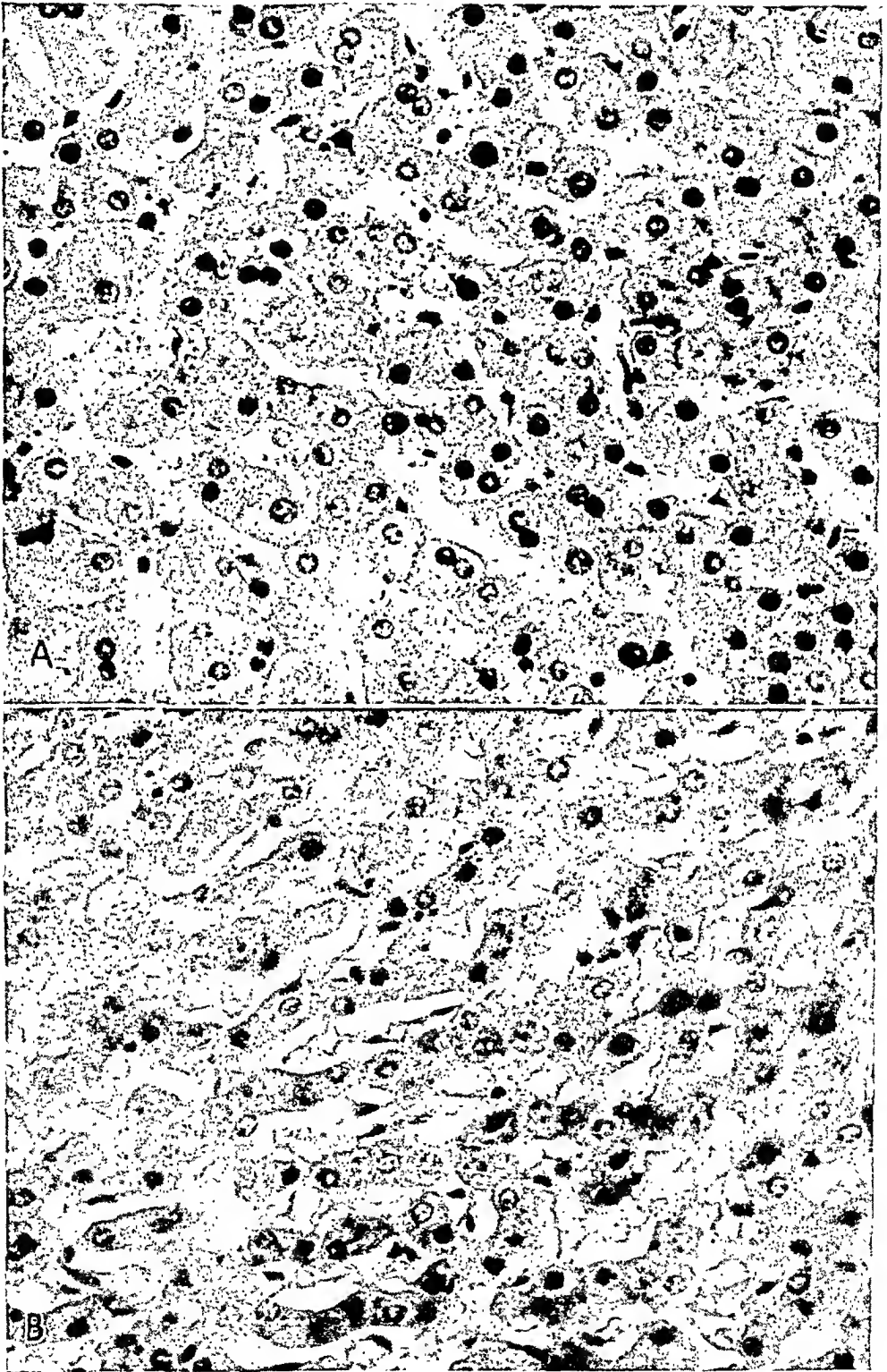


Figure 2
(See legend on opposite page)

tender and had a sharp edge. The spleen was easily palpable. Five hours after aspiration biopsy, signs of shock with anemia developed, and the patient died within thirteen hours after the biopsy. At the autopsy, performed seven hours after death, the abdomen contained large amounts of liquid and clotted blood; the total amount was estimated at 2,500 cc. Three small perforations on the surface of the reddish brown liver led into a 5 cm. long channel, at the end of which a laceration of a large tributary of the portal vein was found. Histologically (fig. 1 *A*), the biopsy specimen revealed a well preserved lobular pattern; however, the arrangement of the liver cell cords was somewhat irregular. The liver cells varied in size; the cytoplasm was finely granular, rich in glycogen, rarely clumped, and occasionally some bile pigment granules or medium-sized fat droplets were seen. The nuclei varied markedly in size, and some were ballooned. Nowhere were liver cells isolated. The bile capillaries were narrow, and communications between them and the perisinusoidal space were not made out. The latter was in most places obliterated and was represented by a small slit containing some albuminoid material only in the center of the lobule. The

Relation of the Width of the Perisinusoidal Space to the Duration of the Agonal Period and the Cause of Death

Cause of Death	Instantaneous Death, Cases in Which Perisinusoidal Spaces Were			Sudden Death, Cases in Which Perisinusoidal Spaces Were		
	Obliter- ated	Partially Open	Open	Obliter- ated	Partially Open	Open
Crash or blast.....	26	1	1	1	1	8
Injuries of the brain.....	32	6	7	3	6	54
Cardiac tamponade and hemorrhage.	21	1	1	2	9	22
Heart failure.....	0	0	0	3	1	54
Suffocation.....	0	0	0	2	0	32
Strangulation.....	0	0	0	1	8	10
Drowning.....	0	0	0	0	0	38
Total.....	79	8	9	12	25	218

sinusoids contained a moderate amount of erythrocytes and also some white cells. Infiltration with round and few polymorphonuclear cells was found in the portal triads. In the autopsy specimen (fig. 1 *B*) the glycogen content of the liver cells was reduced and their eosinophilia increased in comparison with the biopsy specimen; the nuclei appeared not changed. The liver cell cords revealed marked dissociation; the rounded individual cells were in most places separated from each other. Consequently, in many places the bile capillaries appeared ruptured, and some bile seemed to leak through funnel-shaped communications from the bile capillaries into the perisinusoidal spaces. The latter were markedly widened and filled with albuminoid debris. Some sickled erythrocytes were noted in the larger vessels, but rarely in the sinusoids.

Fig. 2.—Hematoxylin-eosin stain. *A*, liver of a soldier dying instantaneously in a crash (Army Institute of Pathology negative 93993). The lining of the sinusoids is adherent to the liver cell cords, and no perisinusoidal space is visible.

B, liver of a soldier dying from an injury of the head more than one hour after the accident (Army Institute of Pathology negative 93964). The perisinusoidal spaces are wide and filled with albuminoid debris.

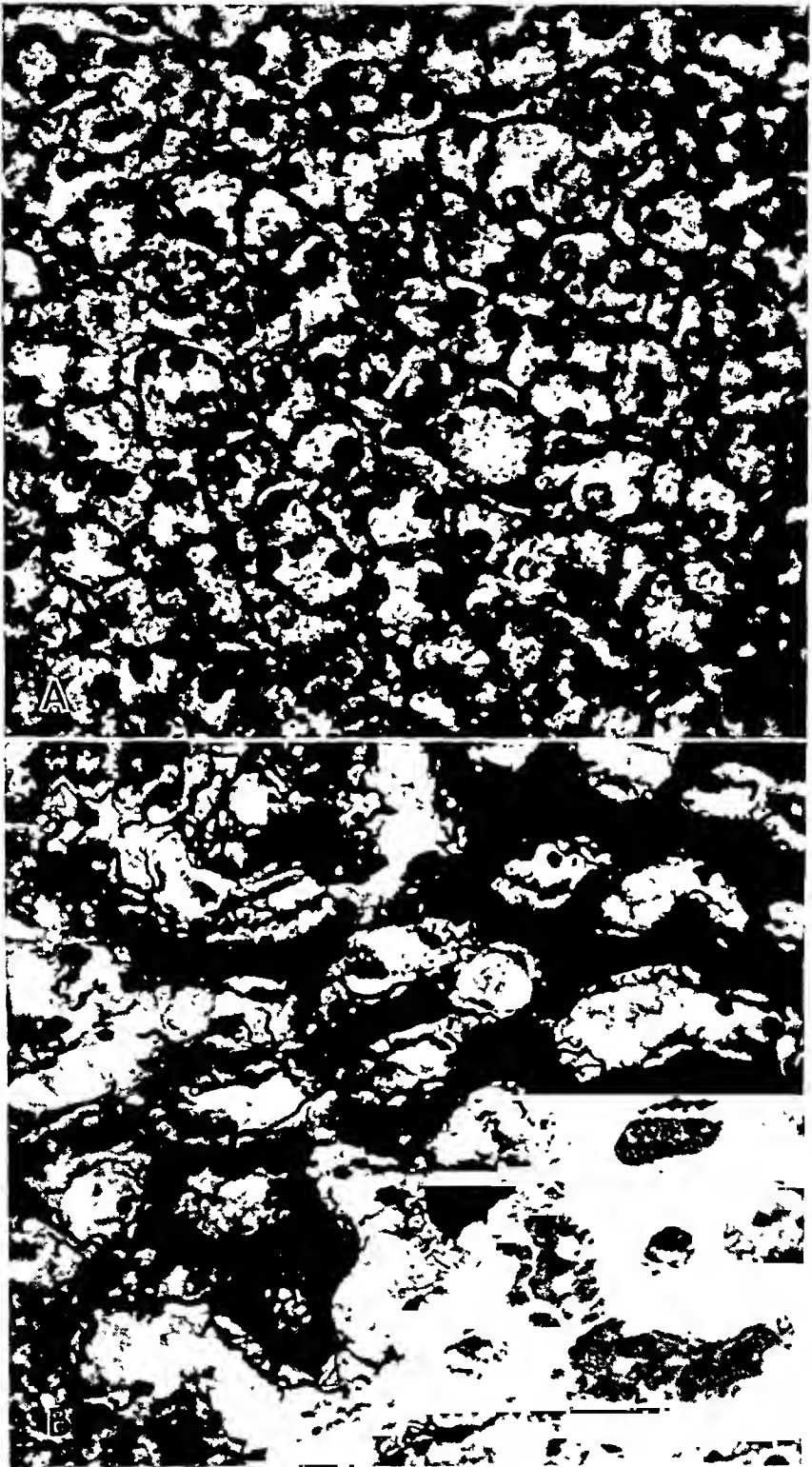


Figure 3

(See legend on opposite page)

Similar differences between autopsy and biopsy specimens were apparent in the 3 other cases of this series; however, more outspoken pathologic changes somewhat obscured the picture.

Influence of the Duration of the Agonal Period on the Histologic Appearance of the Liver.—Some minor alterations from the norm were found in the studied cases of soldiers dying suddenly, such as irregularly scattered fat droplets or wear and tear pigment in the form of branched coarse brown granules. The main emphasis, however, was laid in this study on the relation between the wall of the sinusoids and the liver cell cords. In one group (fig. 2 *A*), the sinusoidal lining was closely attached to the liver cell cords, and no perisinusoidal spaces were visible. The wall itself appeared rather thin in sections stained with hematoxylin and eosin and as a fine blue line in sections stained with aniline blue and the Masson stain. The reticulum fibers were represented by one solid string adherent to the wall of the sinusoids (fig. 3 *A*). Cross fibers were impregnated only in places. They were best seen in the vicinity of the portal and central fields, where they connected with dense collagenous connective tissue. In another group of livers, with hematoxylin-eosin stain a cleft (fig. 2 *B*) containing albuminoid debris was visible between liver cell cords and perisinusoidal lining. It varied but was more noticeable in the central part of the lobule. The Kupffer cells, though separated from the liver cell cords, were not active. The sinusoidal wall was indicated in connective tissue stains by a distinct blue strand, clearly separated from the liver cell cords. Some fine fibers seemed to traverse the perisinusoidal space, and the connective tissue framework was better stained than in the first group. In silver impregnations the long fibers parallel to the axis of the liver cell cords appeared split into several thin fibers with fine clefts between them. A dense net of arcuated or interlaced cross fibers extended from the capillary walls to the liver cell cords. As a whole, more of the reticulum framework was impregnated, owing to its expansion. Between those two groups transition occurred, and consequently a third group was established in which the perisinusoidal spaces were in places open, with expanded reticulum framework, and in others absent.

The perisinusoidal spaces were obliterated in the great majority of the cases of instantaneous death, in 82.3 per cent, and only in a small number were they partially (8.3 per cent) or completely open (9.4 per cent) (table). On the other hand, the perisinusoidal spaces were open throughout in the great majority of cases (83.5 per cent) in which death was sudden but the agonal period of longer duration than 10 minutes, and only in a small number (5.3 per cent) obliterated.

The greatest number of exceptions to this rule occurred in injuries of the central nervous system, which included crash or gunshot trauma of the brain. It was rather difficult to ascertain how quickly death developed in these instances. Death was delayed in a relatively large number of them; it was in this group

Fig. 3.—Silver impregnation of reticulum fibers. *A*, liver of a soldier dying instantaneously in a crash (Army Institute of Pathology negative 93948). The reticulum framework is represented by stringlike axial fibers, whereas cross fibers are hardly recognized.

B, liver with severe hepatic edema from a patient with nephrosis (Army Institute of Pathology negative 93947). The expanded reticulum framework is represented by split axial fibers and many cross fibers extending through the wide perisinusoidal space.

that fairly often the perisinusoidal spaces were only partially opened. Far more uniform were the findings in instances in which a sudden crash, the result of an airplane accident or a grenade explosion, involved the greater part of the body. In such instances, when the patient survived for a while, the spaces were usually open. The tissue spaces were also uniformly obliterated in instantaneous death the result of rapid exsanguination produced by laceration of the heart or a large vessel due to a gunshot or a stab wound. When an extensive hemorrhage caused hemothorax or hemoperitoneum and death was sudden but delayed for more than 10 minutes or even up to 1 hour, the tissue spaces were completely or at least partially open.

Under heart failure were listed instances of coronary thrombosis, interstitial myocarditis without known preceding symptoms, dissecting aneurysm and the like. In some cases of sudden cardiac failure the autopsy failed to reveal the actual cause. The tissue spaces were very wide, especially in the central portion of the lobule, in almost all cases of this group in which circulation stopped at least 10 minutes after the onset of the heart failure. Evidence of acute passive congestion could sometimes be seen. Instances of subacute or chronic congestion were eliminated. Open and occasionally wide tissue spaces were observed in cases of strangulation, despite the rather sudden death. The same was found in a group under the heading of suffocation, in which death was due to pressure placed on the thorax in an accident, to illuminating gas intoxication mostly on a suicidal basis, to cyanide and strychnine intoxication, to anaphylaxis, to lightning, to electrocution and, in a number of cases, to heat stroke. The open and rather wide tissue spaces were filled with granular albuminoid material. The perisinusoidal spaces were at least partially open and often rather wide also in cases of drowning. In some cases of the latter, signs of decomposition were noted, but the spaces were wide even with intact protoplasmic and nuclear staining.

In 13 of the cases just described, alcoholic intoxication contributed to the cause of the fatal accident but did not influence the relation between the speed of death and the width of the tissue spaces. They were closed in 2 instances of instantaneous death and open in the remaining cases.

Little information as to the cytoplasmic structure could be obtained from this material, because the fixation was not uniform. Glycogen stains were not studied; if a fine, regular granulation or vacuolation of the cytoplasm of the liver cells may be considered an indication of the presence of glycogen and the dark homogeneous cytoplasm of the narrower liver cells an indication of its absence, one finds closed tissue spaces, as characteristic of instantaneous death, usually associated with high glycogen content. Partially or fully open tissue spaces as seen in sudden, but not instantaneous, death were observed with both high and low glycogen content. This indicates that open tissue spaces may occur in the presence of swollen liver cells.

In the livers of 160 soldiers dying longer than 24 hours after the onset of their fatal illness, the perisinusoidal spaces were wide open except for 2 instances in which there was diffuse fatty metamorphosis. The width of the perisinusoidal spaces and the deposition of albuminoid material depended on the underlying disease. The distribution and extension of the hepatic edema found in different diseases confirmed the observations of Keschner and Klemperer.¹¹ Marked widening of the perisinusoidal spaces was usually associated with thickening of

11. Keschner, H. W., and Klemperer, P.: *Arch. Path.* 25:583, 1936.

the lining of the sinusoids in hematoxylin-eosin sections; the wall revealed a double contour. With fiber stains the widening appeared to be caused by a pushing apart of the axial reticulum fibers, between which fine clefts were visible. With silver preparations the reticulum network was especially clear in instances of hepatic edema (fig. 3 B).

COMMENT

The presented observations reveal that marked alterations occur in the liver during the agonal period which should be taken into consideration in correlating clinical with histologic observations. The comparing of autopsy and biopsy material as such does not permit differentiation between agonal and postmortal changes. This is better accomplished by studying autopsy material after various durations of the agonal period. The most conspicuous cytoplasmic changes occurring in the agonal period are the well known disappearance of the cytoplasmic glycogen and the subsequent darkening of the cytoplasm in hematoxylin-eosin sections. Other cytoplasmic changes may depend on nutrition and other physiologic factors about which in the cases studied no information was available. Less well appreciated is the fact that dissociation of the liver cell cords with isolation of individual liver cells may be the result of agonal or of postmortal processes. Such changes occurring in autopsy material should only with great caution be correlated with any intravital process. I myself have been guilty of omitting this caution.¹² The point is important in the explanation of the genesis of jaundice. Eppinger¹³ has claimed that regurgitation jaundice the result of damage of liver cells or of biliary obstruction is due to rupture of the bile capillaries in the centers of the liver cell cords with formation of funnel-shaped communications between them and the perisinusoidal spaces. In biliary obstruction the dilated ramifications of the bile capillaries between the liver cells approach the perisinusoidal space and are supposed to rupture into it. The parenchymatous type of jaundice was explained by communications between bile capillary and tissue space produced by a break-up of the liver cell cords. Studies of biopsy specimens, however, fail to support Eppinger's intriguing explanation of regurgitation jaundice, since the assumed communication is not found, as pointed out by Roholm and Iversen.¹⁴ The present study, also, does not favor this explanation, since the dissociation, which forms the basis of this theory, may represent an agonal or a postmortal process. The contention that regurgitation jaundice is associated with

12. Kirshbaum, J. D., and Popper, H.: *Arch. Int. Med.* **65**: 465, 1940.
Steigmann, F.; Popper, H., and Meyer, K. A.: *J. A. M. A.* **122**:279, 1943.

13. Eppinger, H.: *Die Leberkrankheiten*, Berlin, Julius Springer, 1937.

14. Roholm, K., and Iversen, P.: *Acta path. et microbiol. Scandinav.* **16**: 427, 1939.

changes in the smallest bile ducts appears to be more justifiable than the belief that it is associated with a hepatocellular process, as recently emphasized by Watson and Hoffbauer.¹⁵

The fact that central necroses are rarely observed in biopsy material despite their common occurrence in autopsy specimens of similar nature may suggest that they, too, may develop in the agonal period. However, more extensive observations are necessary to confirm this hypothesis.

Significant and so far apparently not appreciated changes of the connective tissue framework and the perisinusoidal spaces occur in the agonal period. The spaces seem, as a rule, to be closed during life, and hepatic edema is a rare occurrence on the basis of this observation. It is common in autopsy material. It develops within a few minutes, as judged from a comparison of the livers of previously healthy soldiers who died instantaneously and those of others who died after a short agonal period. Dilatation of the perisinusoidal spaces of the liver, which is usually associated with accumulation of albuminoid material, has been considered as a morphologic sign of damage of the sinusoids. As a result, protein normally retained within the capillary bed escapes into the perisinusoidal space and binds water. Protein passes through the capillary wall more readily in the liver than in other organs. Roessle¹⁶ considered the escape of protein as inflammatory in nature and spoke of serous hepatitis. He expressed the belief that the edema fluid may elicit connective tissue formation and thus considered the phenomenon as a potential initial stage of cirrhosis of the liver. Subsequently, this concept was elaborated on, and serous inflammation in general was considered an important basic phenomenon.⁹ Serous hepatitis was supposed to be the morphologic substrate of early damage of liver cells and an important feature of parenchymatous hepatitis. Bloom and Maximow,¹⁷ however, denied the existence of these spaces and emphasized that the lymphatic channels reach only into the portal and central fields. Keschner and Klemperer,¹¹ investigating the significance of the widening of the perisinusoidal spaces, assumed two factors: (1) a hydromechanic one (mechanical edema) due to circulatory failure and (2) increased permeability of the sinusoidal wall (primary edema) occurring in various morbid conditions. These authors considered the term "serous hepatitis" as inappropriate.

15. Watson, C. J., and Hoffbauer, F. W.: *Ann. Int. Med.* **25**:196, 1946.

16. Roessle, R.: *Entzündungen der Leber*, in Henke, F., and Lubarsch, O.: *Handbuch der speziellen pathologischen Anatomie und Histologie*, Berlin, Julius Springer, 1930, vol. 5, pt. 1.

17. Bloom, W., and Maximow, A. A.: *A Textbook of Histology*, ed. 4, Philadelphia, W. B. Saunders, 1944.

According to the studies presented here, a short agonal period suffices to produce a significant degree of hepatic edema. It cannot be decided whether this is caused by hydromechanic pressure due to failure of the right side of the heart or by increased permeability due to anoxemia, to which the liver is sensitive. The latter explanation is supported by the rapid development of edema in drowning or suffocation, although acute heart failure may produce it quickly, too. The marked widening of the perisinusoidal spaces in drowning could also be explained by postmortal osmotic changes due to diffusion of water. Hepatic edema was observed by Eppinger⁹ to occur in persons executed by hanging, and Keschner and Klemperer¹¹ recorded it as occurring after suicidal strangulation.

The rapid development of hepatic edema throws some doubt on its physiologic significance in general and the possible sequelae ascribed to it. It surely speaks against an inflammatory character of this process and against the use of the term "serous hepatitis." The fact that some degree of hepatic edema is found in every person who dies not too suddenly weakens conclusions as to the relation of certain diseases to hepatic edema. Moreover, in contrast to assumptions based on the study of autopsy material, it is absent in many instances of severe hepatic damage studied and cannot, therefore, be considered as a significant factor in these diseases. In some conditions associated with infections and intoxications, the presence of marked toxic edema is not questionable; this is often associated with edema of the gallbladder bed.¹⁸

Parallel with the widening of the perisinusoidal spaces, the reticulum framework also expands, leading to better visualization of its cross fibers. In contrast with the kidney (in which the reticulum fibers originate from the basement membrane of the tubules and run toward the capillary wall), the fibers in the liver originate from the lining of the sinusoids and run toward the liver cells, the latter having no continuous basement membrane. Most textbook pictures are based on autopsy material, for example, of executed persons, in which, owing to the edema, the fibers are widely expanded. As a further expression of this expansion of the framework in severe edema, the capillary wall may widen as a result of separation of the axial reticulum fibers; this is outspoken when marked edema with much albuminoid material in the tissue spaces compresses the sinusoidal lumen. One could connect the escape of protein into the perisinusoidal space in severe hepatic edema with the thickening of the wall of the capillary produced by the separation of the reticulum fibers, similar to the association of the thickening of the basement membrane of the renal glomerulus with marked albuminuria in nephrosis.¹⁹

18. Popper.⁵ Keschner and Klemperer.¹¹

19. Bell, E. T.: *Renal Diseases*, Philadelphia, Lea & Febiger, 1947.

The widening of the tissue space seems to precede cytoplasmic changes, such as loss of glycogen or of basophilia. Occasionally, the tissue spaces were open when glycogen still caused the liver cells to swell. This speaks against a mere mechanical opening of the tissue spaces by shrinkage of the liver cells due to loss of glycogen. That mechanical factors may influence the width of the tissue spaces is shown by their obliteration in fatty metamorphosis. However, the latter may even interfere with circulation of the blood.²⁰

It appears from this study that the condition of the tissue spaces may permit conclusions as to the duration of the agonal period in cases of sudden death. This information may sometimes have medicolegal significance.

SUMMARY

A general comparison of the histologic appearances of the liver in biopsy and autopsy specimens reveals, in addition to cytoplasmic differences—caused primarily by the absence of glycogen from autopsy specimens—that the perisinusoidal tissue spaces are usually closed in biopsy specimens and open in autopsy specimens. Open spaces are associated with an extended reticulum framework such that the cross fibers of the latter are better visualized.

A comparison of a biopsy specimen taken from a liver a few hours before death and an autopsy specimen of the same liver shows that striking dissociation of the liver cell cords may occur in the agonal period. Since this is rarely seen in biopsy specimens, even in cases of severe damage of the liver, regurgitation jaundice cannot be explained by communications between bile capillaries and perisinusoidal spaces which are the result of this dissociation. Whereas in livers of persons dying instantaneously the tissue spaces are obliterated, they may be wide open after an agonal period of longer than 10 minutes—for instance, if sudden death results from suffocation, strangulation or heart failure. This observation speaks against the clinical significance of the hepatic edema of serous hepatitis seen in autopsy specimens, the more so since it is usually absent from biopsy specimens, even in the presence of severe damage of the liver.

The condition of the tissue spaces may be helpful in estimating the duration of the agonal period in cases of sudden death.

20. Baxter, J. M.: *Federation Proc.* 7:145, 1948.

MECHANISMS OF LEUKOPENIA WITH INFLAMMATION

An Additional Leukopenic Factor Found in Alkaline Exudates

VALY MENKIN, M.D.

PHILADELPHIA

IN PREVIOUS studies I¹ have shown that there is present in inflammatory exudates a leukocytosis-promoting factor (abbreviated as the L.P.F.). This factor is recovered from the pseudoglobulin fraction of exudates.² Recently it has been found to be distributed between the α_1 and α_2 globulins of exudates.³ The present scheme of extraction has been described in recent publications.⁴ The active group in the leukocytosis-promoting factor of exudates is presumably a polypeptide group attached to the whole globulin molecule.⁵ This group splits off from the rest of the molecule on aging of the material containing the leukocytosis-promoting factor. The leukocytosis-promoting factor helps to explain the mechanism of the leukocytosis frequently associated with inflammation.

The material when freshly extracted from exudates is thermolabile, and it is soluble in an aqueous medium. The object of this communication is to show that when the leukocytosis-promoting factor has been aged for several months it presumably denatures spontaneously and, as a consequence, becomes insoluble in distilled water or saline solution. On centrifugation of the preparation, the supernatant phase is found to contain the split-off leukocytosis-promoting factor, whereas the insoluble part, on the contrary, contains a leukopenic component. The opposing effects elicited by these two fractions result in relative inactivity of the aged leukocytosis-promoting material. Furthermore, it has been often found that during the procedure by which the leukocytosis-promoting material is fractionated from exudates, a fraction that is discarded to obtain a more effective product contains the same leukopenic com-

From the Agnes Barr Chase Foundation for Cancer Research, Temple University School of Medicine.

This investigation was aided in part by a grant from the National Advisory Cancer Council, United States Public Health Service.

1. Menkin, V.: *Am. J. Path.* **16**:13, 1940.

2. Menkin, V.: *Arch. Path.* **30**:363, 1940.

3. Dillon, M. L.; Cooper, G. R., and Menkin, V.: *Proc. Soc. Exper. Biol. & Med.* **65**:187, 1947.

4. Menkin, V.: (a) *Arch. Path.* **41**:376, 1946; (b) *Lancet* **1**:660, 1947.

5. Menkin, V.: *Blood* **3**:939, 1948.

ponent which is found in abundance in aged leukocytosis-promoting material.^{4b} Evidently aging the material produces more of this seemingly denatured leukopenic component. Finally, whole exudate when usually alkaline in nature contains this same leukopenic property in the initial stages of its action after it has been administered to normal dogs. This leukopenic component is thermolabile in contrast to the thermostable leukopenic factor previously described and found also, as a rule, in greater quantities in acid exudates.⁶

TABLE 1.—*Effect of Freshly Prepared Leukocytosis-Promoting Factor on the Number of White Blood Cells*

No. and Date of Preparation of L.P.F.	Dog	Date L.P.F. Was Administered and Amount	White Cell Count Before Injection of L.P.F.	White Cell Counts at Approximate Intervals After L.P.F. Was Injected into Circulation				
				1 Hr.	2 Hr.	3 Hr.	4 Hr.	5 Hr.
58-T of 5/ 1/47	11-T	5/ 2/47—42 mg.	11,150	12,450	15,450	28,150	20,500
51-T of 4/22/47	8-D	4/25/47—44 mg.	8,775	14,100	15,450	17,000	11,150	10,850
30-T of 1/29/47	16-T	1/29/47—25 mg.	12,075	19,900	16,500	22,500	32,000
Average.....			10,667	15,483	15,800	22,550	21,217

TABLE 2.—*Effect of Aged Leukocytosis-Promoting Factor on the Number of White Blood Cells*

No. and Date of Preparation of L.P.F.	Dog	Date L.P.F. Was Administered and Amount	White Cell Count Before Injection of L.P.F.	White Cell Counts at Approximate Intervals After Aged L.P.F. Was Injected into Circulation				
				1 Hr.	2 Hr.	3 Hr.	4 Hr.	5 Hr.
58-T of 5/ 1/47	8-D	10/17/47—138 mg.	9,450	1,050	2,850	5,700
51-T of 4/22/47	60-T	11/11/47— 50 mg.	11,650	11,350	9,950	10,700	17,700	12,050
30-T of 1/29/47	8-D	11/14/47— 53 mg.	8,200	6,300	10,050	7,700	8,300	11,000
Average.....			9,767	6,233	10,000	7,083	13,000	9,583

EXPERIMENTAL PROCEDURE

Dogs are used, and 1.5 cc. of turpentine is injected into the right pleural cavity as described in an earlier communication.⁷ Within about twenty-four hours an acute inflammation usually develops. The exudative material withdrawn from such a cavity is, as a rule, alkaline in nature. The leukocytosis-promoting substance is extracted from the exudative material as described previously.⁴ The substance is soluble in isotonic sodium chloride solution, and in this solvent it is injected in varying concentrations into the hearts of normal dogs. The results of three such experiments are assembled in table 1. When the leukocytosis-promoting extract is kept at room temperature in a desiccator under phosphoric anhydride for several months, a change in some of its properties occurs, and it becomes relatively inactive. The now aged leukocytosis-promoting substance is found insoluble in an aqueous medium, and it is biologically relatively inert. In fact, it may in certain instances give rise to an initial leukopenia, which is detectable

6. Menkin, V.: Arch. Path. **41**:50, 1946.

7. Menkin, V.: Am. J. Path. **10**:193, 1934.

in the first hour after its administration. Observations relating to this are summarized in table 2, and the course of an individual experiment is graphically illustrated in chart 1. It is quite clear that there is a marked difference between the effect of the freshly prepared leukocytosis-promoting factor and that of the aged preparation.

When aged leukocytosis-promoting material is suspended in an aqueous medium and the suspension is subsequently centrifuged, the supernatant phase is seen to contain the active leukocytosis-promoting factor. In earlier studies this has been shown to be referable to a simple polypeptide which splits off from the rest of the globulin molecule during the probable process of denaturing as the material ages.⁵ The observations relating to this appear in table 3. It is seen that with the exception of two experiments there is no initial leukopenic tendency after administration of this supernatant fraction. The average of seven experiments fails to show any initial drop in the total white cell count

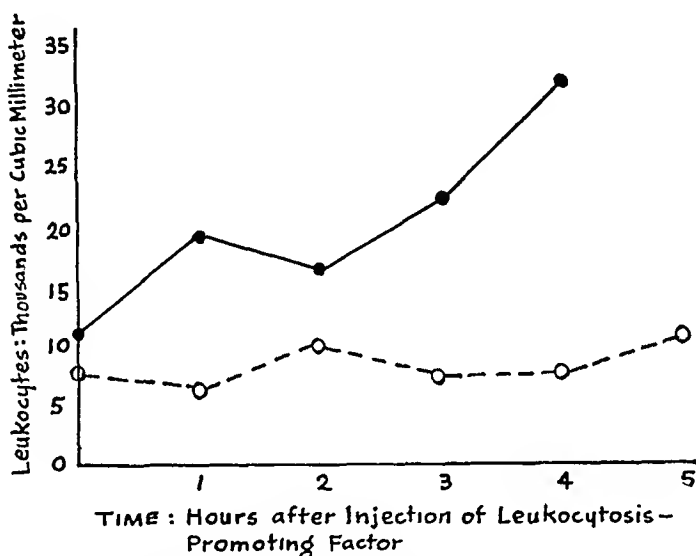


Chart 1.—Effect of aging of the leukocytosis-promoting factor. The solid line represents the number of leukocytes per cubic millimeter of blood following injection of a fresh preparation of the leukocytosis-promoting factor; the broken line, the number following injection of the aged preparation.

(table 3). On the contrary, when the residual or precipitated material from aged leukocytosis-promoting material is studied, it is seen that there is a leukopenic component especially conspicuous during the first hour after administration of this insoluble precipitate (table 4). The dissociation is not perfect, and after an interval of about two or three hours the original level of circulating leukocytes is restored and actually begins to rise as in the case of the supernatant phase (compare tables 3 and 4). Nevertheless, it is this initial leukopenic tendency on the part of the precipitate of the aged leukocytosis-promoting factor that seems to counteract the opposed activity exerted by the supernatant fraction.

The question immediately arises whether this leukopenic component present in the precipitated fraction of aged leukocytosis-promoting material is similar in character to the leukopenic factor described to be present in appreciable amounts in acid exudates.⁶ The leukopenic factor of acid exudates is thermostable, and it is closely linked to pyrexin, from which it can in turn be dissociated by

TABLE 3.—*Effect of Supernatant Fraction (Probably Polypeptide in Nature) from Aged Leukocytosis-Promoting Factor on the Number of Circulating Leukocytes*

Dog and Date	Amount of Dried Supernatant Fraction Injected, Obtained from Aged L.P.F.	White Cell Count Before Injection of Supernatant Fraction	White Cell Counts at Approximate Intervals After Supernatant Fraction Was Injected into Circulation					
			1 Hr.	2 Hr.	3 Hr.	4 Hr.	5 Hr.	6 Hr.
63-T 10/23/47	17 mg. from aged L.P.F. of 58-T of 5/1/47	7,250	12,400	15,150	17,650	13,300
11-T 10/24/47	50 mg. from aged L.P.F. of 58-T of 5/1/47	12,600	11,500	21,950	17,800	10,950
52-T 10/30/47	38 mg. from aged L.P.F. of 58-T of 5/1/47	5,450	8,700	11,250	16,400	12,650
54-T 11/ 5/47	27 mg. in normal canine blood serum from aged L.P.F. of 58-T of 5/1/47	7,100	9,200	8,900	15,450	15,300	15,950
31-T 11/ 6/47	36.5 mg. in normal canine serum from aged L.P.F. of 58-T of 5/1/47	7,550	7,300	11,600	13,350
63-T 11/10/47	31.9 mg. in normal canine serum from aged L.P.F. of 58-T of 5/1/47	12,750	8,600	12,450	18,150	22,150	18,350
60-T 11/12/47	5 cc. of fluid from aged L.P.F. of 51-T of 4/22/47	9,750	12,250	10,500	13,700	15,000	19,200	21,750
Average.....		8,921	9,993	13,114	16,071	16,392	17,833

TABLE 4.—*Effect of Precipitated or Leukopenic Component from Aged Leukocytosis-Promoting Material on the Number of Circulating Leukocytes*

Dog	Amount of Suspension of Precipitate* from Aged L.P.F.	White Cell Count Before Injection of Precipitated Component of Aged L.P.F.	White Cell Counts at Approximate Intervals After Precipitated Component Was Injected into Circulation						
			1 Hr.	2 Hr.	3 Hr.	4 Hr.	5 Hr.	6 Hr.	7 Hr.
63-T	— cc.† from 58-T of 5/1/47	13,150	3,650	10,450	10,950
54-T	3.5 cc. from 58-T of 5/1/47	7,300	4,900	4,850	8,200	10,150	9,250	15,000	11,750
8-D	7.0 cc. from 58-T of 5/1/47	5,250	2,550	4,600	8,150	6,400
60-T	10-11 cc. from 51-T of 4/22/47	10,450	7,200	7,850	10,600	10,900
11-T	15 cc. from 30-T of 1/29/47	13,400	8,050	12,600	12,550	9,850	12,600
Average.....		9,910	5,270	8,110	9,963	9,250	10,075	13,800

* The precipitated component was suspended in saline solution.

† The actual amount was not recorded.

incomplete hydrolysis with tenth-normal hydrochloric acid.⁶ Under such circumstances the potency of the leukopenic factor remains essentially unaltered.⁶ In the present study the leukopenic component of aged leukocytosis-promoting material is inactivated by incomplete hydrolysis with tenth-normal hydrochloric acid. The observations relating to this are shown in table 5. It is evident from table 5 that with the exception of two questionable experiments the leukopenic component of aged leukocytosis-promoting material is wholly inactivated by incomplete acid hydrolysis. The average figures indicate that there is no initial leukopenia following administration of the product of incomplete hydrolysis of aged leukocytosis-promoting material. This finding points out the difference in the two separate leukopenic components obtained from exudative material. Furthermore, the results obtained are not referable to the partial hydrolysis in itself, for when the process of hydrolysis is performed with tenth-normal hydrochloric

TABLE 5.—*Inactivation of the Leukopenic Effect from the Precipitated Component of Aged Leukocytosis-Promoting Material by Incomplete Hydrolysis of That Component*

Dog and Date	Amount of Suspension of Incompletely Hydrolyzed Precipitate from a Given Amount of Aged L.P.F.	White Cell Count Before Injection of Incompletely Hydrolyzed Precipitated Component of Aged L.P.F.	White Cell Counts at Approximate Intervals After Incompletely Hydrolyzed Precipitated Component Was Injected into Circulation					
			1 Hr.	2 Hr.	3 Hr.	4 Hr.	5 Hr.	6 Hr.
46-T 12/ 4/47	10 cc. from 86 mg. of 58-T of 5/1/47	8,675	15,700	17,600	14,800	12,900	15,800
60-T 12/ 5/47	7 cc. from 60 mg. of 58-T of 5/1/47	16,525	15,900	20,350	14,850	18,950
63-T 12/ 6/47	12 cc. from about 45 mg. of 51-T of 4/22/47	11,800	11,500	15,200	19,600
54-T 12/ 8/47	10 cc. from about 44 mg. of 51-T of 4/22/47	10,450	14,900	14,400	15,200	14,200	15,550	14,200
8-D 12/10/47	14 cc. from 44 mg. of 58-T of 5/1/47	8,783	9,200	11,250	9,863	10,000	9,975
54-T 12/11/47	19 cc. from 140 mg. of 51-T of 4/22/47	13,583	18,850	13,300	16,000	13,900
Average.....		11,636	14,342	15,350	15,093	13,170	12,763	16,317

acid only, there is no essential change in the number of circulating leukocytes on injection of such material.⁶ The exclusive leukocytosis following partial hydrolysis of the precipitated component of aged leukocytosis-promoting material is doubtless referable to the heat-stable polypeptide in the aged leukocytosis-promoting factor. This factor is usually found in the supernatant phase, but it can also be present to some extent in the precipitated part of the aged material (table 4).

With the foregoing finding that a leukopenic component is present in the insoluble part of aged leukocytosis-promoting material, the question arises as to the presence or the absence of such a leukopenic factor in freshly withdrawn whole exudate. When such an exudate is injected into the circulation of a normal dog, there is initial leukopenia, soon followed by leukocytosis. The latter is referable to the leukocytosis-promoting factor present in the exudate.¹ At the time that the leukocytosis-promoting factor was first demonstrated to exist in exudates, it was pointed out that at first there is transitory leukopenia.¹

This state of affairs recalled the leukopenia described by Ewing⁸ and by Webb⁹ which occurs during anaphylactic shock. Several years later a similar leukopenic phase was said to occur with improperly purified leukocytosis-promoting factor.¹⁰

TABLE 6.—*The Possible Presence in Exudates of a Thermolabile Factor, Besides the Thermostable Leukopenic Factor, Capable of Lowering the Number of Circulating Leukocytes*

Dog	pH of Exudate	Amount of Exudate Injected into the Circulation, Cc.	White Cell Count Before Injection of Exudate	White Cell Count at Approximately One Hour After the Injection of the Exudate
26-D.....	7.4	5	10,500	6,550
52-D.....	7.5	9-10	8,175	3,700
Administered in dried form in saline solution, this being the equivalent of 514 mg. of lyophilized exudate				
60-T.....	7.2	4	15,300	12,450
52-D.....	7.5	5	15,350	9,450
				2 hours after the injection of the exudate
54-T.....	7.2	4	9,900	6,150
87-T.....	7.5	4.5	9,225	6,000
87-T.....	7.5	5	9,350	7,550
87-T.....	7.5	4.5	6,075	6,950
				2 hours after the injection of the exudate
87-T*.....	7.5	2	7,550	10,550
Average.....	10,158	7,773
				Per cent reduction=23.5
22-D†.....	7.5	5	19,375	16,000
				2 hours after injection of the boiled exudate
60-T†.....	7.2	4.5	19,900	18,550
63-T†.....	7.2	4	12,675	12,400
87-T†.....	7.5	5	10,825	7,150
85-T†.....	7.5	5	12,900	10,000
91-T†.....	7.5	4.5 to 5	15,400	13,200
91-T†.....	7.5	5	15,675	13,100
Average.....	15,279	12,914
				Per cent reduction=15.5
63-T†.....	6.0	3	11,625	8,150
87-T.....	6.0-6.2	5	9,250	7,050
94-T†.....	6.0	5	11,350	9,450
Average.....	10,742	8,217
				Per cent reduction=23.5
54-T§.....	6.0	3	13,675	3,800
91-T†.....	6.0-6.2	5	11,050	10,050
85-T§.....	6.0	4.5	11,500	8,150
Average.....	12,075	7,333
				Per cent reduction=39

* Any leukopenic effect was possibly masked by the effect of the leukocytosis-promoting factor in the sample of exudate.

† This sample of exudative material was brought to the boiling point before being injected into the circulation of the animal.

‡ This sample of exudate was removed at postmortem examination from the chest of an animal that had been given a second injection of the irritant and as a consequence was very ill.

§ The foregoing explanation holds true of the material injected, but in addition it was boiled.

8. Ewing, J.: New York M. J. 61:257, 1895.

9. Webb, R. A.: J. Path. & Bact. 27:79, 1924.

10. Menkin, V., and Kadish, M. A.: Am. J. M. Sc. 205:363, 1943.

At that time this was thought to be due to some toxic impurities.¹⁰ In the present study exudative material, some at alkaline and some at acid p_H , was injected into dogs, and thereafter the number of leukocytes per cubic millimeter of blood was studied. It was generally found that when an alkaline exudate (p_H ranging from 7.2 to 7.5) was introduced into the blood of a normal dog, an hour later a drop in the number of circulating leukocytes tended to occur. This reduction averaged 23.5 per cent (table 6). When, however, such exudate was heated to the boiling point, the reduction in white cells was distinctly less, averaging 15.5 per cent. It seems as if heating had destroyed a part of the leukopenic component present in the alkaline exudate. When the exudate was in an acid phase (p_H 6.0 to 6.2), its introduction was likewise followed by an initial drop in

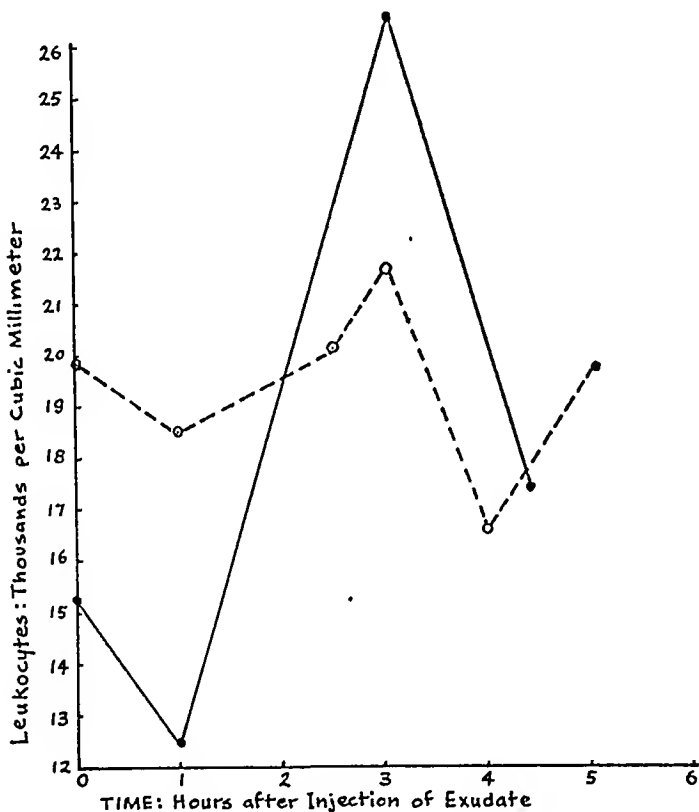
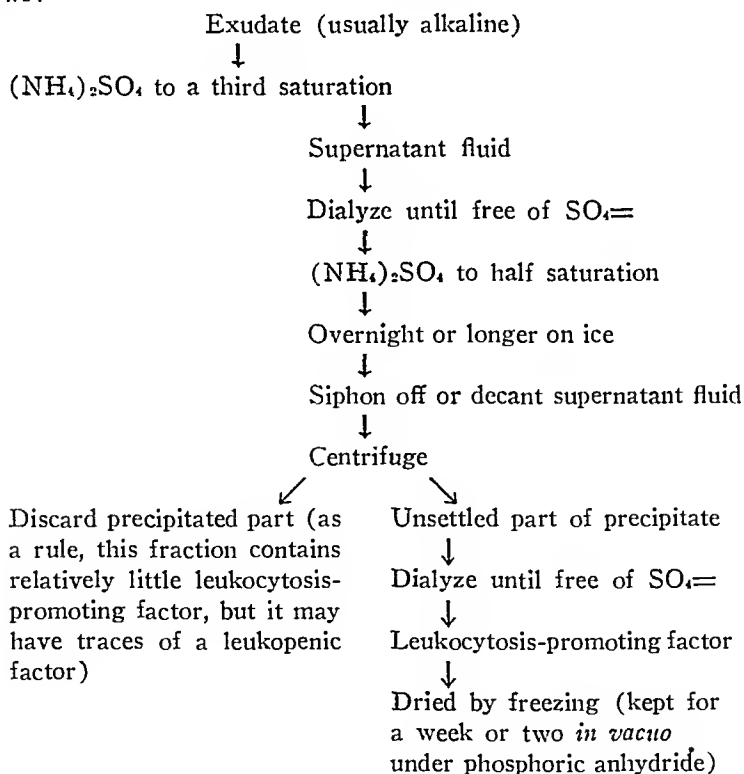


Chart 2.—Effect of boiling of alkaline exudate on the leukopenic components. The solid line represents the number of leukocytes per cubic millimeter of blood following injection of unheated exudate; the broken line, the number following injection of boiled exudate.

the leukocytic level, averaging also 23.5 per cent (table 6). Bringing such acid material to a boil, however, rendered the exudate even more potent as a leukopenic agent. The average reduction now amounted to 39 per cent (table 6). What is the interpretation of these results? It seems as if an alkaline exudate may contain two leukopenic factors. One is thermolabile and the other, probably in smaller quantities, is thermostable. The latter does not appear to be different from the leukopenic factor described in an earlier study.⁶ When the alkaline exudate is boiled, the thermolabile factor is inactivated, leaving only the thermostable leukopenic factor. This would account for the reduction in the drop in the white blood cell count from 23.5 to 15.5 per cent, i. e., the reduction

obtained by simply bringing the alkaline exudate to a boil (table 6). On the other hand, an acid exudate contains the heat-stable leukopenic factor usually found in abundance in such exudates, whereas the thermolabile leukopenic factor is present, if at all, in insignificant amounts in acid material. Boiling the acid exudate inactivates the leukocytosis-promoting factor present, since the latter tends to be thermolabile.¹ The consequence is the unobstructed action of the thermostable leukopenic factor, so that the drop of the white blood cell level is even more marked, with a reduction of 39 per cent (table 6). The initial drop in the circulating white cells is shown in chart 2. Subsequently a rise occurs, due to the leukocytosis-promoting factor present in the exudative material (chart 2). Boiling such material and injecting it are followed by an initial drop which is definitely less pronounced than that observed with the untreated exudate (chart 2). The effect of any leukocytosis-promoting factor is essentially eliminated by heating the exudate. The exudative material utilized in this particular experiment was alkaline (p_H 7.2) (table 6; chart 2). In conclusion, the effects obtained with the two types of exudates studied seem to be referable to the respective differences in the concentrations of the thermolabile and the thermostable leukopenic component.^{10a}

The present scheme for the extraction of the leukocytosis-promoting factor is as follows:



10a. Differential absorption by boiling exudates of different initial p_H is an improbable interpretation of the aforesaid results, for in earlier studies it was shown that there is a leukopenic factor which is a heat-stable polypeptide obtained usually at acid p_H and associated with pyrexin,⁶ whereas the present factor is usually obtained from alkaline exudates as a thermolabile factor associated with the globulins of these exudates.

It is essential to perform proper lyophilization for about 24 hours. Longer periods may end to denature the material, i. e., as far as biologic potency is concerned. It is best to freeze the material as a fairly thin film in a glass container. Finally, it has been observed that insufficient lyophilization, resulting in thawing, followed then by a second attempt to dry the material, usually yields a relatively inactive product with a leukopenic phase in it. It seems from these remarks that the leukocytosis-promoting factor of exudates is easily denatured and thus may lose its potency if proper precautions are not followed.

As indicated in the scheme of extraction, the final precipitated fraction sometimes contains a leukopenic factor similar to the one found in abundance in aged leukocytosis-promoting material. The observations relating to this appear in table 7. It is quite clear that in 8 of 9 experiments the initial leukopenic

TABLE 7.—*Leukopenic Effect Induced by the Precipitated Fraction Obtained in the Final Extraction of the Leukocytosis-Promoting Material*

Dog	Amount of Precipitated Material Dissolved in an Aqueous Medium	White Cell Count Before Injection of Material	White Cell Counts at Approximate Intervals After Precipitate Fraction Was Injected Into Circulation					
			1 Hr.	2 Hr.	3 Hr.	4 Hr.	5 Hr.	6 Hr.
8-D	27 cc. (equivalent to 20 cc. of original exudate)	14,913	8,625	12,350	15,150	17,850
31-D	22 cc. (80 mg. of material)	14,725	6,950	6,850	10,100	12,100	13,750	17,650
22-D	11 cc.	14,050	21,150	5,050	7,350	6,150	5,750
8-D	15 cc. (equivalent to 10 cc. of injected exudate)	13,175	11,100	11,900	18,500	11,100	16,000	15,800
31-D*	11 cc.	18,325	12,000	22,250	23,900	26,150	27,200	28,450
45-D*	13.5 cc. (51.5 mg. L.P.F. and precipitate)	17,000	4,550	11,950	14,600	15,900	40,000
26-D*	22 cc. (equivalent to 20 cc. of original exudate)	12,375	4,450	10,350	13,100	15,150	14,150
46-D*	10 cc.	17,050	5,950	20,100	33,900
22-D†	20 cc. (equivalent to ± 18 cc. of original exudate)	20,950	10,550	16,050	18,950	23,900	18,700	20,550
Average.....		15,907	9,481	12,983	17,283	16,038	19,364	20,613

* This dog received the precipitated fraction along with some leukocytosis-promoting factor.

† This dog received, not the precipitated fraction, but rather an initial fraction in the preparation of the leukocytosis-promoting factor. Both the precipitated fraction and the leukocytosis-promoting factor are presumed to have been still present in it.

tendency on the part of this precipitated fraction was present. It is for this reason that in the extraction of an active leukocytosis-promoting factor the precipitate is being discarded (see extractive scheme). This leukopenic fraction extracted from exudates is thermolabile; boiling the material inactivates it (table 8). It is thus seen that this fraction obtained from fresh exudates does not seem to differ in any way from the fraction obtained from the precipitate of the aged leukocytosis-promoting extract (compare table 4 with table 7). It is therefore my belief, in view of these observations, that in alkaline exudates there is a thermolabile leukopenic factor closely associated with the globulins of the leukocytosis-promoting factor. It seems to occur as a consequence of a denaturation of the proteins. At first it occurs with injury to cells, so that it can be recovered in an alkaline exudate. Eventually more of it forms as the leukocytosis-promoting extract is allowed to age for several months. The material, kept in a desiccator under phosphoric anhydride, apparently denatures

spontaneously, loses its original property of being soluble in an aqueous medium, and evidently forms this leukopenic factor in greater abundance. The latter can be recovered as the insoluble part of aged leukocytosis-promoting material. For this thermolabile leukopenic factor, which is found also in alkaline exudates, though to a less extent perhaps, the term "leukopenin" is suggested to distinguish

TABLE 8.—*The Thermolability of the Precipitated Fraction Obtained in the Extraction of the Leukocytosis-Promoting Factor*

Dog	Initial White Cell Count	White Cell Count One Hour After Intravenous Injection of Precipitated Fraction (Heated to Boiling)
84-T*	14,075	14,400
91-T*	13,425	12,350
87-T.	6,450	10,050
94-T.	10,025	13,100
85-T.	11,175	11,850
Average.....	11,030	12,750

* The precipitated fraction was refluxed for about 10 minutes with tenth-normal hydrochloric acid, neutralized with normal sodium hydroxide, and dialyzed to rule out the presence of the leukopenic factor.

TABLE 9.—*Initial Effect of Leukocytosis-Promoting Factor* Obtained from Human Exudate on the Number of Circulating Leukocytes*

Dog	Number of Leukocytes per Cubic Millimeter of Blood Prior to Injection of Material	Initial Drop in White Cell Count Following Injection of Material
7-31.....	9,900	4,000*
7-31.....	11,650	2,650 (Essentially no euglobulin admixed to L.P.F.)
7-31†.....	18,200†	4,500†
7-33§.....	15,900	12,000†
7-29.....	18,400	4,700 (Essentially no euglobulin fraction in this material)
7-34§.....	7,150	5,000 (Essentially no euglobulin fraction in this material)
Average.....	13,533	5,475

* This leukocytosis-promoting fraction of human exudate contained the entire globulin content of the exudate. (The method utilized has been described by Menkin and Kadish.¹⁰)

† Fifty cubic centimeters of whole exudate was used instead of the leukocytosis-promoting extract.

‡ This figure is a first approximation inasmuch as it was interpolated from a graphic representation of these experiments.

§ The leukocytosis-promoting material from a human source was injected subcutaneously; in other experiments it was injected intravascularly.

it from the leukopenic factor which is closely associated with pyrexin and which is thermostable.⁶

In a recent discussion Hadfield and Garrod¹¹ expressed the view that in the conclusions drawn by me there may be an element of generalization; perhaps a

11. Hadfield, G., and Garrod, L. P.: Recent Advances in Pathology, ed. 5, Philadelphia, The Blakiston Company, 1947.

similar attitude has been pointed out by Wilson.¹² These views do not seem wholly warranted, for leukotaxine has been found in the exudates of several different species of animals.¹³ The leukocytosis-promoting factor has been identified in dogs,¹ rabbits¹⁴ and man.¹⁵ Finally, necrosin has been studied both in dogs and in man.¹⁶ Nevertheless, in view of these criticisms a study was made in an endeavor to find out whether leukopenin was also present in another species, namely, man. An initial leukopenic tendency had been noted previously as occurring both in human exudate and in a leukocytosis-promoting extract of human exudate.¹⁰ The observations relating to this are assembled in table 9, and the course of an experiment with human material is graphically illustrated

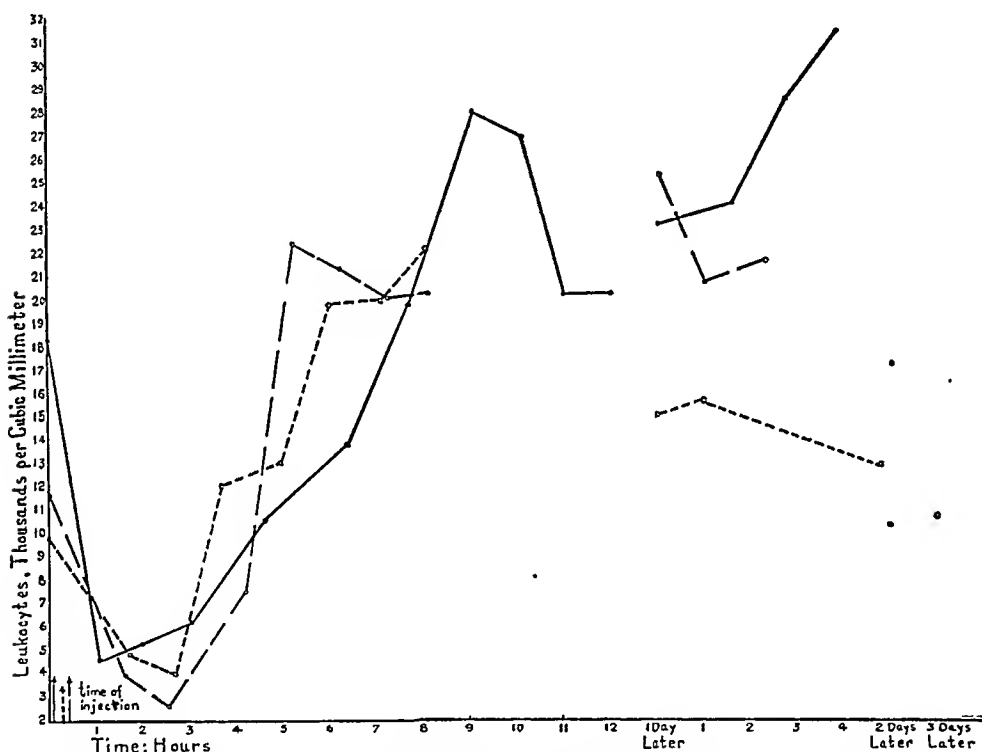


Chart 3.—Effect of the human leukocytosis-promoting factor. One curve (—) represents the number of leukocytes per cubic millimeter of blood of a dog (7-31) after the dog was given an injection of whole exudate (50 cc.) obtained from a patient; a second curve (— — —), the number after the dog was given a desiccated leukocytosis-promoting extract (obtained from approximately 31 cc. of the whole exudate); a third curve (— · — · —), the number after the dog was given 100 mg. of the desiccated extract.¹⁰

in chart 3. It is quite clear from the data that a similar leukopenic trend is found soon after administration of human exudate or of an extract of it con-

12. Wilson, G. S.: Principles of Bacteriology and Immunity, ed. 3, Baltimore, Williams & Wilkins Company, 1946.

13. Menkin, V.: Dynamics of Inflammation, New York, The Macmillan Company, 1940. Cullumbine, H., and Rydon, H. N.: Brit. J. Exper. Path. **27**:33, 1946

14. Menkin, V.: Proc. Soc. Exper. Biol. & Med. **64**:448, 1947.

15. Menkin, V.; Kadish, M. A., and Sommers, S. C.: Arch. Path. **33**:188, 1942.

16. Menkin, V.: Arch. Path. **36**:269, 1943.

taining the leukocytosis-promoting factor. These results, in addition to the ones obtained with other substances from exudates, would somewhat weaken the contention of excessive generalization based on only meager factual information.

It is also of interest to know the type of cells affected by the leukopenin recovered from alkaline exudates. In the initial drop following the intravascular injection of leukopenin the mononuclear cells are primarily involved. This includes a combination of both the lymphocytes and the monocytes. In a few instances one of these two types of cells is more involved than the other; but in general both lymphocytes and monocytes are affected, i. e., in over 80 per cent of the cases studied. In about 50 per cent of the observations the polymorphonuclear neutrophils are also involved. The fall does not seem to affect the immature, or one lobe, neutrophils. These cells steadily increase in number after the injection of the material. Finally, it can be stated here, also, that the drop in the number of circulating leukocytes following the injection of leukopenin is not referable to a redistribution effect, for the same drop occurs in a sample of cardiac blood as well as in one taken at the periphery by nicking a vessel of the ear.

Chemical determinations were made on a sample of the precipitate of relatively fresh leukocytosis-promoting extract and on one of aged L. P. F. The two determinations showed essentially no difference, as indicated in table 10.

TABLE 10.—*Chemical Determinations*

	Precipitate of Relatively Fresh Leukocytosis-Promoting Extract (About 3 Wk. Old)	Precipitate of Aged Leukocytosis-Promoting Extract (About 15 Mo. Old)
Nitrogen, per cent.	10.55	11.46
Carbon, per cent.	43.88	46.44
Hydrogen, per cent.	6.85	7.40
Sulfur, per cent.	0.51	1.37
Phosphorus, per cent.	0.33

Except perhaps for the slight rise in sulfur concentration, aging the leukocytosis-promoting extract failed to alter materially the constituents of the precipitate studied, as is indicated in table 10.

COMMENT

These studies indicate that exudative material contains at least two factors concerned in the mechanisms of leukopenia with inflammation. There is in alkaline exudates, associated with the globulins of the leukocytosis-promoting factor, a thermolabile leukopenic factor. This leukopenic component appears to be a product of protein denaturation concomitant with cell injury, for it seems to be found in appreciable quantity by merely allowing an active preparation of leukocytosis-promoting factor, at first devoid of it, to age for several months.¹ A spontaneous denaturation occurs. The leukocytosis-producing extract becomes insoluble in an aqueous medium, and in the precipitated part the leukopenic component can be recovered. With fresh exudates the component is found present in an unused fraction obtained in the preparation of an active leukocytosis-producing extract. The chemical

constituents of the insoluble fraction of aged leukocytosis-promoting extract and the unused fraction obtained at the time of recovery of fresh leukocytosis-promoting extract are essentially similar. The term "leukopenin" is suggested for this leukopenic component identified particularly in alkaline exudates.

In alkaline exudates there is also present the thermostable leukopenic factor previously described.⁶ In such exudates it is recovered in relatively small quantities. It is found, however, in larger abundance in acid exudates. This is not fully surprising, for the latter leukopenic component has been found to be closely associated with pyrexin, the pyrogenic factor. Pyrexin, in turn, has been found to occur more frequently and in larger amounts in acid than in alkaline exudates.¹⁷

Whether leukopenin acts by trapping the leukocytes in various tissues is yet to be determined.¹⁸ The reduction of the number of circulating white cells seems to affect primarily the mononuclear cells, namely, the lymphocytes and the monocytes. Sometimes one type of these cells is more involved than the other type, but in general both types seem to be affected. The polymorphonuclear leukocytes are depressed in number during the leukopenic phase caused by leukopenin in only about 50 per cent of the cases. Further study is necessary to determine the exact mechanism of the reduction involved.

Finally, to the other substances listed in an earlier communication,¹⁹ liberated by injured cells as a result of their impaired biochemistry, leukopenin can also be added. This factor, in addition to the leukopenic factor of exudates, helps in one's understanding of the mechanisms of leukopenia with inflammation. None of these substances is present in normal blood serum.²⁰

17. Menkin, V.: *Federation Proc.* **4**:149, 1945.

18. Menkin, V.: *Arch. Path.* **42**:154, 1946.

19. Menkin, V.: *Science* **105**:538, 1947.

20. The view has been expressed that the present and the previous substances isolated from exudates are crude mixtures and therefore that their meaning may be somewhat doubtful. This type of criticism fails to take into consideration certain facts. The whole exudate, as such, is shown to possess certain biologic properties. These properties are not present in normal blood serum. Further analysis of the whole exudate yields biochemical units which have precisely the same properties as the whole exudate. To be certain, it would be desirable to know the exact chemical formulation of these units. It is hoped that future studies will yield such data; but present investigators do not seem quite ready for such studies; or at least these have not yet been undertaken by specialized chemists. This has always been the history of almost any biologic substance derived either from animal or from plant sources. At first the substance is obtained in a relatively crude form. Subsequently it is purified to its final form. The studies on the leukocytosis-promoting factor have progressed somewhat in this direction, for the active grouping has already been ascertained to be a relatively simple polypeptide.

SUMMARY AND CONCLUSIONS

There is present in exudates, particularly those that are alkaline in nature, a leukopenic component closely associated with the globulins of the leukocytosis-promoting factor. This leukopenic component is thermolabile. This distinguishes it from the thermostable leukopenic factor previously described as recovered from acid exudates.⁶ The leukopenic component of exudates associated with the globulins of the leukocytosis-promoting factor seems to be a product of a protein denaturation following the initial injury of cells with the onset of inflammation. Aging the leukocytosis-promoting factor induces the further production of this leukopenic component, presumably by spontaneous denaturation. The formation of this factor tends to reduce the potency of, or even to inactivate, the usual leukocytosis-promoting factor, the factor which accelerates the discharging of polymorphonuclear leukocytes into the blood stream. To render the leukocytosis-promoting factor extracted from freshly withdrawn exudates more effective, the leukopenic component, particularly that found in alkaline exudates, is eliminated in the scheme of extraction of the leukocytosis-promoting factor.^{4b}

Earlier studies have demonstrated that a thermostable leukopenic factor is present in exudates.⁶ The knowledge that the thermolabile leukopenic component of exudates is in combination with the thermostable one helps in one's understanding of the mechanisms of leukopenia with inflammation.

The initial leukopenia induced by the thermolabile leukopenic component of exudates affects primarily the mononuclear type of white cells and to some extent the polymorphonuclear leukocytes.

The term "leukopenin" is suggested for this additional leukopenic component concerned in the mechanism of leukopenia with inflammation.

SIGNIFICANCE OF THE BETA GRANULES IN THE ISLETS OF LANGERHANS OF THE PANCREAS

S. S. BARRON, M.D.

MINNEAPOLIS

THE ISLETS of Langerhans are composed of two distinct types of cells, alpha and beta cells, which may be distinguished by appropriate staining. My associates and I have used Gömöri's stain exclusively, since it is easy to apply and gives consistent results. With this stain the alpha cells are colored red, and their cytoplasm shows no distinct granules; but the beta cells contain numerous small blue particles, which are called beta granules (fig. 1).

It is now well established that insulin is formed by the beta cells. Injections of alloxan cause complete necrosis of all the beta cells without injury to the alpha cells, and the animal becomes permanently diabetic. The adenoma of the pancreas which causes hyperinsulinism is composed of cells which contain some beta granules.

Beta granules are greatly reduced in number or are entirely absent in the human diabetic pancreas in about two thirds of the cases. Degranulated beta cells of the human pancreas show no other evidence of injury, and the significance of degranulation is not understood.

The purpose of this investigation was to determine whether the beta cells may be degranulated by experimental procedures which decrease the demand for insulin, such as fasting, feeding a diet consisting exclusively of fat or daily administration of insulin.

Best, Haist and Ridout,¹ by means of extraction and quantitative assays, found definite reductions in the insulin content of the pancreas of rats after subjecting these animals to fasting, after keeping them for a period on a fat diet and after administration of insulin. They pooled the pancreases of 10 normal rats and found the total insulin content to be 26.5 units. After a fasting period of seven days the insulin content of the pancreas of 10 animals was 14.1 units. Rats fed a normal diet and given a daily injection of protamine zinc insulin showed an insulin content of 10 units per 10 rats after a period of seven days.

From the Department of Pathology, University of Minnesota.

This investigation was supported by a grant made to Dr. E. T. Bell by the Office of Naval Research.

1. Best, C. H.; Haist, R. E., and Ridout, J. H.: Diet and the Insulin Content of the Pancreas, *J. Physiol.* **97**:107, 1939.

Rats fed a diet 90 per cent fat for seven days showed an insulin content of 12.3 units per 10 pancreases. But when insulin was administered daily to rats on the fat diet, the insulin content was reduced to 3.8 and 4.1 units per 10 pancreases after a period of seven days.

These investigators expressed the belief that under these experimental conditions the reductions of insulin were due to decreases of demand occasioned by the lack of carbohydrate and by the exogenous supply of insulin, respectively. The islets were put at rest and apparently formed less insulin. In one experiment they found a decrease of beta granules in pancreases with a low content of insulin, suggesting

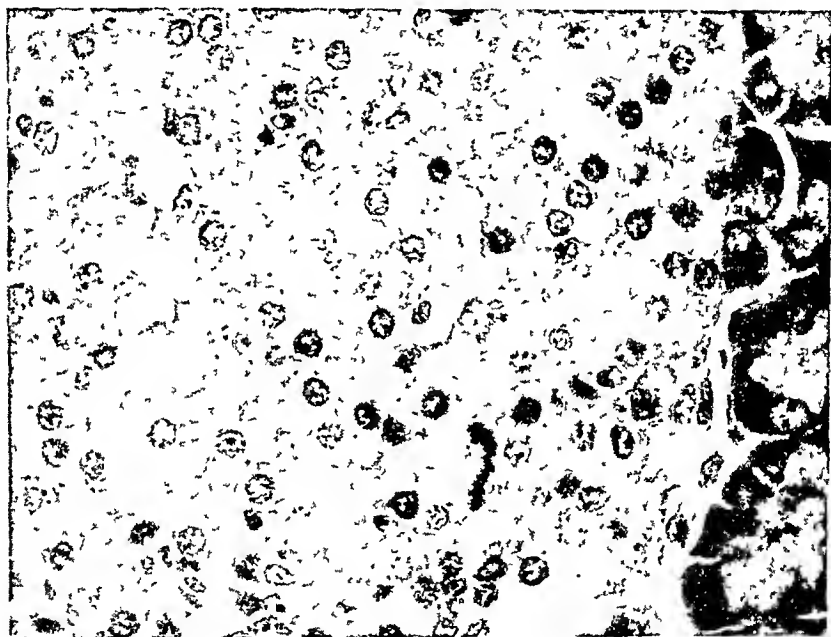


Fig 1—Islet of Langerhans from the pancreas of a normal rat, stained by Gomori's method. The beta cells are filled with small granules, which are colored a deep blue in the original preparation.

a direct correlation of beta granules and insulin content. However, to demonstrate beta granules, these investigators used Bowie's stain, which is not as satisfactory as Gomori's stain.

MATERIAL AND METHODS

White rats were used for all of the experiments of the present study, and the animals were divided into four groups. Group 1 consisted of fasted rats; group 2, of rats maintained on lard; group 3, of rats fed olive oil exclusively, and group 4, of rats fed a regular diet and given a daily injection of protamine zinc insulin. The pancreas was fixed in Bouin's fluid, and paraffin sections were stained by Gomori's method.

Group 1. Fasting Rats (table 1).—The 13 rats of this group were kept in separate cages and were allowed a free supply of water but were given no food. A number of rats were lost because they died during the night and the subsequent postmortem changes prevented a histologic study of the pancreas. Only those animals are included which were killed at the end of the experimental period.

TABLE 1.—*Fasting Rats*

Serial No.	Initial Weight, Gm.	Final Weight, Gm.	Duration, Days	Beta Granules
47-62.....	215	...	3	3
47-63.....	275	...	5	3
47-64.....	270	...	5	2
47-65.....	270	...	5	3
48-209.....	160	95	8	0
48-208.....	215	120	8	1
48-206.....	155	110	8	2
48-205.....	120	85	8	1
48-201.....	190	119	8	3
48-202.....	175	103	8	1
48-203.....	160	100	8	1
48-207.....	160	105	8	2
48-210.....	160	95	8	3

It will be noted in table 1 that there was a marked loss of body weight. The numerals 0 to 3 indicate the amount of beta granulation. Grade 0 means that no granules were demonstrable, and grade 1 indicates a striking reduction of granules. Grade 2 means a moderate decrease of granules within the limits of normal

TABLE 2.—*Rats Fed on Lard*

Serial No.	Initial Weight, Gm.	Final Weight, Gm.	Duration, Days	Beta Granules
47-154.....	230	160	13	1—
47-155.....	185	110	13	1—
47-156.....	200	130	13	1—
47-157.....	210	145	13	0
47-158.....	230	170	13	1—
47-159.....	225	160	13	1—
47-160.....	150	100	13	0
47-161.....	125	75	13	1—
47-162.....	125	75	13	0
47-163.....	125	80	13	1—
47-164.....	140	85	13	1—
47-165.....	125	75	13	1—
48-211.....	155	105	9	1—
48-212.....	150	100	9	0
48-213.....	120	80	9	0
48-214.....	170	125	9	1
48-215.....	210	115	9	0
48-216.....	160	110	9	1
48-217.....	185	135	9	1—
48-218.....	175	135	9	1
48-219.....	140	95	9	1—
48-220.....	170	115	9	1

variation, and grade 3 indicates normal granulation. A fasting period of five days or less did not affect the beta granules, but a fast of eight days caused complete degranulation in 1 rat and marked reduction of granules in 4 others, but in 4 animals the granules were unaffected by fasting for eight days.

Group 2. Rats Fed on Lard (table 2).—The 22 rats of this group were maintained on a diet consisting exclusively of lard. They had free access to water. Ten animals were killed at the end of a 9 day period, and 12 after 13 days. There was a striking loss of body weight, especially in the group maintained for 13 days. The degree of degranulation was about as marked in the 9 day as in the 13 day group. In 6 animals the beta cells were completely degranulated (grade 0) (fig. 2); in 12 animals there were only occasional beta granules (grade 1—), and in 4 there was definite reduction of granules but it was not so severe as in the others (grade 1).

Best and Haist² found that rats maintained for seven days on a diet 90 per cent fat showed the insulin of the pancreas reduced to about 50 per cent of normal. Possibly the insulin content would have been reduced more if the experiment had been continued longer.

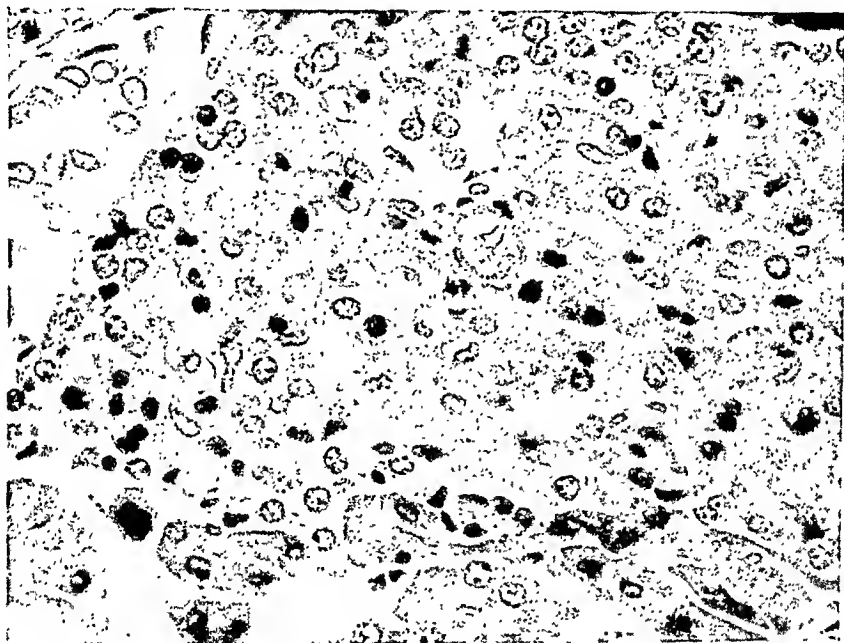


Fig. 2.—Islet of Langerhans from the pancreas of a rat maintained exclusively on lard for 13 days. The beta cells are completely degranulated. Photomicrograph.

Group 3. Rats Fed on Olive Oil (table 3).—The 17 rats of this group were fed olive oil exclusively but were allowed free access to water. Nine of the 17 rats were killed after 20 days of the diet, and the others after 23 days. There was a severe loss of weight in every animal. In 6 rats there was complete degranulation of the beta cells (grade 0), and in 7 others only occasional beta granules were found (grade 1—). In the remaining 4 rats the degranulation was marked but was not so severe as in the others (grade 1).

Group 4. Rats Treated with Insulin (table 4).—The animals of this group were maintained on the regular diet without any restrictions. Each rat was given a daily injection of 4 to 6 units of protamine zinc insulin. The animals ate

2. Best, C. H., and Haist, R. E.: The Effect of Insulin Administration on the Insulin Content of the Pancreas, *J. Physiol.* **100**:142, 1941.

well and showed no clinical disturbances. They either maintained their body weight or grew heavier. Three rats killed after one week showed severe degranulation of the beta cells. In the cases of 2 rats a biopsy of the pancreas made after two months of insulin injections showed complete degranulation of the beta cells. There was no evidence of atrophy or of degeneration of the beta cells after two months, and no indication that this treatment would ultimately produce diabetes.

TABLE 3.—*Rats Fed on Olive Oil*

Serial No.	Initial Weight, Gm.	Final Weight, Gm.	Duration, Days	Beta Granules
47-134.....	175	130	23	1—
47-135.....	130	80	23	0
47-136.....	175	110	23	0
47-137.....	125	65	23	1—
47-138.....	150	105	23	0
47-139.....	155	100	23	1—
47-140.....	150	110	23	1
47-141.....	140	85	23	1—
48-221.....	150	85	20	1—
48-222.....	130	65	20	0
48-223.....	140	80	20	0
48-224.....	130	55	20	0
48-226.....	165	90	20	1
48-227.....	190	105	20	1
48-228.....	185	95	20	1—
48-229.....	135	60	20	1—
48-230.....	200	105	20	1

TABLE 4.—*Rats Treated with Insulin*

Serial No.	Initial Weight, Gm.	Final Weight, Gm.	Duration, Days	Beta Granules
47-186.....	120	...	45	0
47-192.....	170	275	Biopsy, 2 mo.	0
48-232.....	145	135	7	1—
48-236.....	145	140	7	1
48-234.....	140	135	7	0
47-189.....	220	270	Biopsy, 2 mo.	0

SUMMARY

The three experimental procedures outlined, viz., fasting, maintenance on a diet restricted to fat and daily administration of insulin, all produce degranulation of the beta cells. Fasting is less effective in producing degranulation than the other procedures, probably because the animals of this group did not live so long. When there is no carbohydrate in the diet, insulin is not required, and one may believe that insulin is not produced when it is not needed. The work of Best and Haist showed that the insulin content of the pancreas is decreased by the procedures outlined. Since there is a direct correlation between the insulin content of the pancreas and the number of beta granules, the conclusion seems justified that the beta granules represent a precursor of insulin.

NUCLEIC ACIDS AND CYTOLOGIC CHANGES IN REGENERATING RAT LIVER

ROBERT E. STOWELL, M.D., Ph.D.*
KANSAS CITY, KAN.

THERE is considerable recent evidence that the nucleic acids of cells play an important role in the formation of cellular proteins and that the nucleolus with its associated chromatin is one of the vital cell structures participating in this function.¹ To obtain further information concerning these relationships, experiments were undertaken employing liver tissue, which has large nucleoli in cells capable of remarkably rapid regenerative growth. The changes in nucleic acids as determined by special cytochemical and macrochemical methods were correlated with morphologic changes including the mean sizes of the nucleolus, the nucleus and the cytoplasm of the hepatic cell.²

Detailed ultraviolet cytochemical observations³ on a large variety of types of living cells indicate that the nucleolus-associated chromatin (heterochromatin) produces protein substances which constitute, in part at least, the nucleolus. In conjunction with these nucleolar materials the nuclear membrane produces ribose nucleic acids leading to increased cytoplasmic protein. In favorable material a nucleic acid gradient from the nucleolus toward the nuclear membrane as well as from the membrane into the surrounding cytoplasm has been shown. This evidence of the important relationship of ribose nucleic acid in protein synthesis and of the participation of the nucleolus-associated chromatin, the nucleolus and the nuclear membrane is based on many

* Advanced Medical Fellow of the Commonwealth Fund, 1946-1947.

This investigation was aided by grants from the American Cancer Society, the National Cancer Institute, the Karolinska Institutet and the Rockefeller Foundation.

From the Institute for Cell Research, Karolinska Institutet, Stockholm, Sweden, and the Department of Pathology, Washington University School of Medicine, St. Louis.

1. (a) Caspersson, T.: Symp., Soc. Exper. Biol., no. 1, 1947, p. 127. (b) Darlington, C. D.: *Nature*, London **149**:66, 1942; (c) Symp., Soc. Exper. Biol., no. 1, 1947, p. 252. (d) Koller, P. C.: *ibid.*, 1947, p. 270. (e) Schultz, J.: Caspersson, T., and Aquilonius, L.: *Proc. Nat. Acad. Sc.* **26**:515, 1940.

2. Stowell, R. E.: *Am. J. Path.* **23**:883, 1947.

3. (a) Caspersson.^{1a} (b) Hydén, H.: Symp., Soc. Exper. Biol., no. 1, 1947, p. 152. (c) Thorell, B.: *Acta med. Scandinav.* **117**:334, 1947. (d) Caspersson, T., and Santesson, L.: *Acta radiol.*, 1942, supp. 46.

observations on cells of mammals, birds, fish, insects, plants, bacteria and yeasts. The cells represent a wide variety of normal and pathologic conditions of retarded and stimulated growth, including adult, embryonic and neoplastic tissues. However, relatively few observations have been made with these technics on regenerating tissues.

The morphologic changes during liver regeneration have been studied and reviewed by numerous investigators,⁴ who have shown that the liver is capable of replacing two thirds of its volume within a few days after partial hepatectomy. Among the factors that influence the rate of increase of liver volume are species, diet, age, blood flow and degree of partial hepatectomy. Since the liver substance is restored by diffuse hypertrophy and hyperplasia of the remaining parenchymal cells rather than by regrowth from the site of extirpation, from the point of view of experimental morphogenesis the appropriateness of the term "regeneration" may be questioned (Fishback^{4c}; Higgins and Anderson^{4d}; Sulkin^{4e}). However, the term "regeneration" is used more broadly in medicine, and the widely accepted usage of "liver regeneration" contraindicates an attempt to introduce a more precise term, with the inevitable confusion attending it.

Few previous investigators have made quantitative measurements on changes in cellular constituents in the early stages of liver regeneration. Sulkin^{4e} made quantitative measurements of nuclear size after 28 days of regeneration. By this time the liver has almost returned to its normal condition, and one could not expect to learn much of the initial mechanism of the restoration processes. He did observe a mean increase of about 5 per cent in the nuclear diameters of the regenerating cells, twice as many binucleate cells and a greater frequency of polyploidy.

Brues, Drury and Brues^{4a} made counts of the cells of liver at various stages of regeneration and found virtually no increase in number during the first day, while there was a 50 to 60 per cent increase in size. During the second day the weight of the liver increased 44 per cent and the number of cells 64 per cent. On the third day the weight increased less than 10 per cent and the number of cells by 26 per cent. They observed that the mean cell size decreased after cell division was initiated but that the size remained a little larger than the normal for twelve days.

Ferreira^{4b} measured nuclear and nucleolar areas in regenerating and normal rat liver and found that they were largest at the second day. Only 20 cells were measured in a rat at each stage of regeneration.

4. (a) Brues, A. M.; Drury, D. R., and Brues, M. C.: *Arch. Path.* **22**:658, 1936. (b) Ferreira, A. E. M.: *Folia anat. micr. conimb.* **15**:1, 1940. (c) Fishback, F. C.: *Arch. Path.* **7**:955, 1929. (d) Higgins, G. M., and Anderson, R. M.: *ibid.* **12**:186, 1931. (e) Sulkin, N. M.: *Am. J. Anat.* **73**:107, 1943.

The initial rapid increase in liver substance is comparable to the enlargement of a developing 8 to 10 day chick embryo⁵ or to that of a proliferating tissue culture⁶ and greater than that of most cancers.⁷ Such rapidly growing liver tissue should be especially suitable for correlated studies of morphologic changes in cells and in their chemical constituents, including the nucleic acids.

MATERIALS AND METHODS

Young adult white and piebald rats of unselected lineage weighing 200 to 350 Gm. were maintained on a synthetic diet to facilitate comparison of these results with those of other experiments. The composition of the diet was 45 per cent wheat starch, 14 per cent sucrose, 11.5 per cent casein, 0.5 per cent l-cystine, 4 per cent Osborne-Mendel inorganic salt mixture, 5 per cent brewers' yeast, 19 per cent hydrogenated soy bean oil and 1 per cent cod liver oil. To remove the more labile hepatic cellular substances influenced by diet, the rats were initially fasted 20 hours preoperatively and again as nearly a comparable time as the conditions of the experiment would permit before the animal was killed to obtain the regenerating liver. Drinking water was freely available.

With the rat under ether anesthesia, the median and left lateral lobes of the liver were removed according to the technique described by Brues, Drury and Brues.^{8a} Preliminary experiments indicated that 65 per cent of the total liver substance was extirpated by this method. The rats responded well to the operation, so that secondary effects on the liver were considered minimal.

Preliminary observations on regeneration extending to 15 days showed the maximum changes of nucleolar volume at 24 hours. Therefore the changes in regeneration during the first 48 hours were carefully studied at six hour intervals on a total of 13 rats. The early stages of liver regeneration have received relatively little attention from most other investigators.

Liver tissue obtained from each rat at the time of partial hepatectomy and after a determined postoperative period of regeneration was preserved by the Altmann-Gersh⁸ freezing-drying technique and by fixation in Stieve, Carnoy, formaldehyde and Regaud solutions, absolute alcohol and cold acetone. Thus all measurements on regenerating liver could be referred to the normal liver of the same animal and a variety of fixed tissues after comparable treatment were available for special or comparative purposes. Fixation in Stieve fluid, consisting of saturated aqueous mercuric chloride 76 cc., formaldehyde solution U. S. P. 20 cc. and glacial acetic acid 4 cc., for 18 hours was followed by washing in 95 per cent alcohol. Dehydrated tissues were infiltrated with paraffin and sectioned usually at 4 microns thickness.

Morphologic measurements of ratios of tissue space, cytoplasm and nucleus were made by Chalkley's⁹ method for recording ratios of points indicated by ocular pointers. This method is a relatively rapid and accurate means of determining

5. Carrel, A., and Ebeling, A. H.: *J. Exper. Med.* **48**:105, 1928.

6. Murray, H. A.: *J. Gen. Physiol.* **9**:29, 1926.

7. Bashford, E. F.: *Scient. Rep. Imp. Cancer Research Fund* **4**:197, 1911.

8. Gersh, I.: *Anat. Rec.* **53**:309, 1932.

9. Chalkley, H. W.: *J. Nat. Cancer Inst.* **4**:47, 1943.

ratios of different morphologic tissue constituents. For each tissue a total of 800 loci were recorded on a differential blood-counting machine, which is efficiently adaptable for this purpose.

Nuclear and nucleolar diameters were measured in the same plane on 100 hepatic parenchymal cells with an eye piece screw micrometer of the filar type on sections stained by the Feulgen reaction¹⁰ and a light green counterstain. Since the nucleolus is surrounded with chromatin which stains by the Feulgen reaction for thymonucleic acid, the diameter of nucleolar material between and not including the nucleolar associated chromatin was measured. By assuming that the structures were spherical the respective nuclear and nucleolar volumes were calculated from the formula $\frac{4}{3} \pi r^3$. The errors arising from slight deviations from a true spherical shape and from observation in less than maximum diameter were not considered significant, since they are largely obviated by averaging 100 measurements and by using results only for comparative purposes. From these data the mean relative cytoplasmic volume can be calculated by multiplying the mean nuclear volume by the percentage cytoplasmic volume and dividing by the percentage nuclear volume as determined by Chalkley's ratio measurements. Thus relative morphologic data on nucleolar, nuclear, cytoplasmic and cell volume were obtained for each corresponding normal and regenerating liver, and the significance of the results was analyzed statistically.

Absorption curves of small areas of the cytoplasm of hepatic cells were made according to the ultramicrospectrophotometric method developed by Caspersson.¹¹ The absorption of ultraviolet rays at wavelengths ranging from 240 to 350 millimicrons was determined photometrically by measuring and comparing (1) the transmission of monochromatic light passing through a predetermined part of the cell mounted on a special quartz microscope and (2) the blank light transmission adjacent to the tissue. From the calculated extinction coefficients the absorption curves can be computed. A correction for nonspecific light scattering and loss in the tissues can be applied according to Rawleigh's formula. Examples of such spectrophotometric absorption curves are shown in figure 1. In this experiment large numbers of such determinations were not made, so that it was not possible to establish the presence of significant differences in the cytoplasm of normal and regenerating cells by this method. Purines and pyrimidines have a high absorption in the region of 260 millimicrons. Because of their predominant distribution in nucleotides, under the conditions of measurement, the absorption maximum at 260 corresponds well to the concentration of nucleic acids. Proteins have a much less pronounced and less precise absorption in the region of 280 millimicrons. Thus under properly controlled conditions one can study the distribution and concentration of nucleic acids and proteins within cellular parts with an area of 1 square micron. Details of the methods and the apparatus and their accuracy have been published.¹¹

After the ultraviolet ray absorption characteristics of a tissue have been established by the direct photoelectric technic, additional absorption data can be obtained if necessary by a photographic method.^{3a} Tissue sections of 4 microns' thickness mounted on quartz slides in glycerin (specific gravity, 1.25) were photographed with monochromatic light under carefully controlled conditions.

10. Stowell, R. E.: *Stain Technol.* **21**:137, 1946.

11. Caspersson, T.: (a) *Skandinav. Arch. f. Physiol.*, vol. 73, supp. 8: J. Roy. Micr. Soc. **60**:8, 1940; (b) footnote 1a.

The specimens were placed on a quartz microscope employing Zeiss monochromatic lenses corrected for 257 or 275 millimicrons. From a Köhler rotating cadmium spark gap light source monochromatic illumination of 231, 257 or 275 millimicrons was used. By means of a Köhler view finder, which provides a magnification of the image focused on a fluorescent screen, one can select areas suitable for photography and achieve precise focusing. The same area was photographed for comparative absorption measurements at the different wavelengths.

Although numerous precautions were taken to control variable factors in exposing and developing the photographic plates, a densitometric calibration was made on each plate. On a free space adjacent to the image of the tissue photographed, the image of a rotating step sector was recorded. This provided values for 10, 20, 40, 60, 80 and 100 per cent transmission which could be used in calibrating the optical densities of the tissue image. Densities of the photographic plate for the calibration and comparable cell images on corresponding plates were

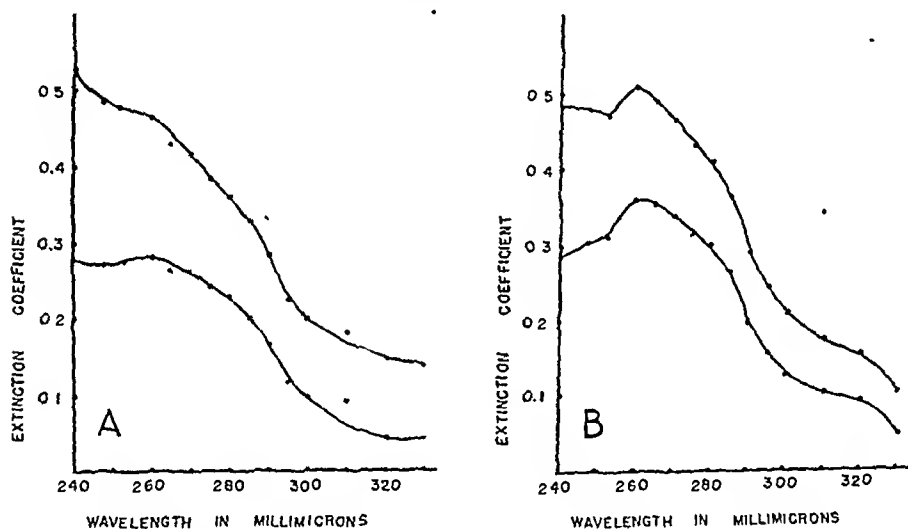


Fig. 1.—Absorption curves of cytoplasm of normal (*A*) and regenerating (*B*) liver cells. The lower curve has been corrected for light scattering and dispersion.

determined with automatic recording microdensitometers of the Leitz or Knorr-Albers type.¹² Light transmission through areas of 0.5 by 1 mm. of the plate was recorded by the densitometer.

By making a continuous densitometric recording across the nucleus and cytoplasm of a cell one obtained a curve as shown in figure 2. Here one can see the correspondence between the high density of such cellular constituents as nuclear membrane and nucleolus and the transmission peaks of the recording. Generally, most normal and regenerating liver cells showed increasing cytoplasmic absorption as one approached the nuclear membrane. Percentage transmission readings were obtained for photographs of the same cells taken at 257 and 275 millimicrons for two corresponding cytoplasmic points taken near but not at the nuclear membrane and for the absorption maximum for the nucleolus. From the corrected transmission readings the optical density or extinction coefficients were calculated for

12. The Aluminum Ore Company, East St. Louis, Ill., allowed us to use their instrument in making many of these determinations.

each wavelength. Duplicate determinations on the same cell in different photographs showed good agreement of densitometric tracings, although the over-all accuracy of this photographic technic is less quantitative than the direct photo-electric method. In instances in which there was considerable difference in the focus of the photographs at 257 and 275 millimicrons, it was found advisable to discard the plates to avoid errors in the results.

RESULTS

Following removal of two thirds of the liver there is a rapid increase in the weight of the remaining tissue, so that in these experiments the original weight was virtually regained by the third day. Examination of sections of normal and regenerating liver from the same animal at six hour intervals showed a decrease in the vascular

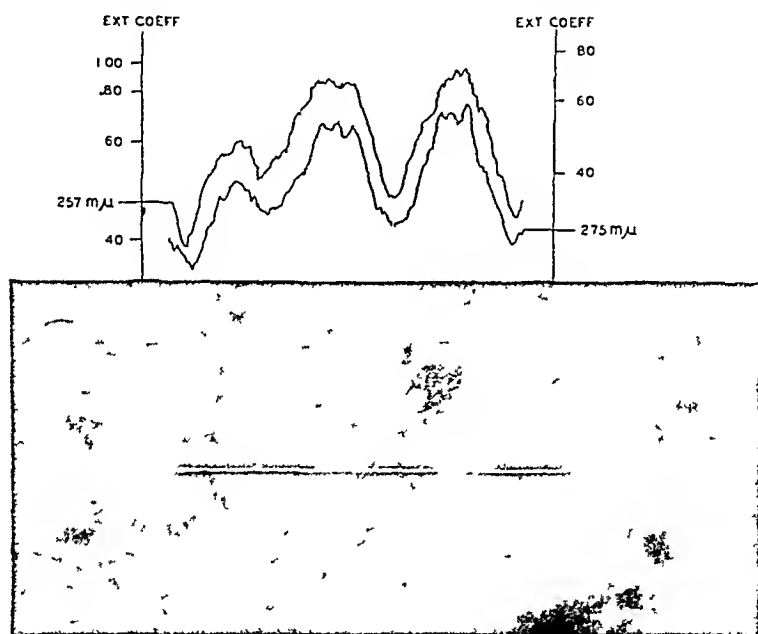


Fig. 2—Densitometric tracings of photographs taken at 257 and 275 millimicrons wavelength. The line on the photograph of the liver cell shows the path of the densitometric recording across cytoplasm, nuclear membrane and two nucleoli. The correspondence of absorption with cell structures is evident, especially at the nuclear membrane and the nucleoli.

spaces and an increase in the size of the regenerating hepatic cells. The cytoplasm of the cells containing an increased amount of vacuolation, which was demonstrated by sudan staining to be chiefly fat. As indicated in table 1, this change was quite evident by 6 hours and was most pronounced at 18 to 24 hours. Mitotic figures were seen at many stages but in these experiments were most frequent at 30 hours.

The measurements of ratios of tissue constituents by Chalkley's method showed that the regenerating liver had percentages of vascular tissues amounting to 31 to 47 per cent of that of the normal. Vascular tissue, which included the vessel wall, the Kupffer cells and the lumen,

comprised an average of 28 per cent of normal liver substance in these animals. Normal rat liver cells were 91 per cent by volume cytoplasm. Since the nucleoli comprised less than 1 per cent of the normal cell volume, it was more accurate to compute their volume on the basis of their diameters. Comparison of nucleolar and nuclear volumes computed from diameters indicates that the nucleoli of normal rat liver cells constitute 0.4 to 0.7 per cent of the nuclear volume. The results of the volumetric measurements are shown in table 2 and figure 3. The values for P in table 2 indicate the probability of obtaining comparable results by chance. Values less than 0.01, which signify that there is 1 chance in 100 of getting the same results by accident, are considered statistically significant. Thus even after 6 to 12 hours of regeneration significant increases in the mean volumes of cytoplasm, nucleus and nucleolus have occurred. Their volumes increase to a maximum of 2.6, 2.2 and 4.1 times at 18, 24 and 24 hours, respectively. Following

TABLE 1.—*Changes Noted in the Early Stages of Liver Regeneration*

Period of Regeneration, Hr.	Wt. of Liver, Percentage	Fat	Mitosis
6	50	++	0
12	50	++	0
18	50	+++	0
24	55	+++	+
30	55	++	+++
36	65	++	++
42	55	++	+
48	85	++	+
72	100	+	0

the increased cell division which begins at 24 hours and reaches a maximum at 30 to 36 hours, the volumes of the regenerating cell and its constituents decrease toward normal. The nucleus shows more pronounced volume changes in regeneration than the nucleolus or the cytoplasm. Observations on young rats, in which regeneration proceeds more rapidly,¹³ indicated increase in nucleolar volume as high as 7.5 times at 24 hours.

The mean coefficient of variation of the normal nuclei was 37 per cent and that of the regenerating nuclei 39 per cent. The nucleolar size during the second day showed more variation than in normal cells, the coefficients of variation being 73 and 94 per cent, respectively.

The number of nucleoli per hundred nuclei were counted. It is difficult to express these results in terms of the complete nucleus since, because of its size, usually all of a nucleus was not included in a 4 micron section. There was an average of 1.4 nucleoli per nuclear section in the normal liver. As shown in figure 3, definite fluctuations

13. Norris, J. L.; Blanchard, J., and Povolny, C.: Arch. Path. 34:208, 1942.

were observed in regenerating cells in the number of nucleoli per nuclear section, in the mean nucleolar mass and in the mean nucleolar mass per nuclear section. The observed changes would not seem to be adequately explained by the possibility that sections of larger nuclei

TABLE 2.—*Mean Morphologic Changes Noted in the Volumes of the Cytoplasm, the Nucleus and the Nucleolus of Regenerating Liver Cells as Compared with Normal Cells of the Same Rat, Expressed as Percentage Increase*

Period of Regeneration, Hr.	Cytoplasm	Nucleus	P	Nucleolus	P
'6	144	120	0.0007	120	0.0168
12	122	122	0.0000	219	0.0000
18	258	140	0.0000	288	0.0000
24	239	217	0.0000	406	0.0000
30	236	186	0.0594	206	0.0000
36	164	133	0.0000	162	0.0004
42	187	134	0.0918	133	0.0382
48	143	147	0.0000	74	0.0003

would show a smaller proportion of the total number of nucleoli within the complete nucleus. Generally, when nucleoli were observed to be larger, they were also found to be fewer. This is corroborated by the results shown in figure 3, where diverse fluctuations in number and size of nucleoli are evident.

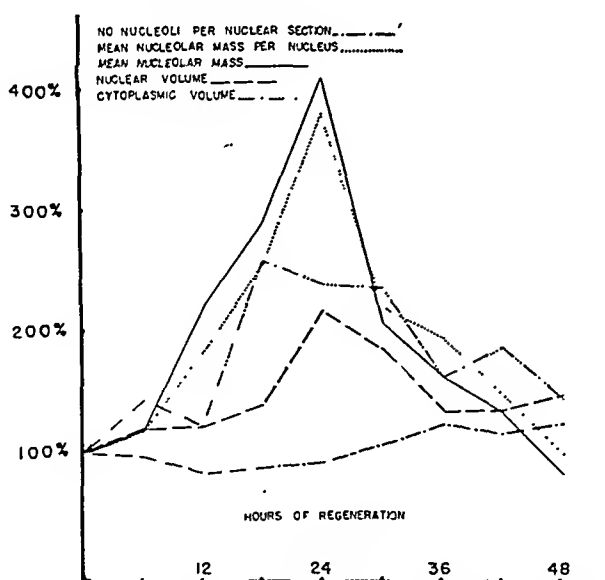


Fig. 3.—Volume of cellular constituents during liver regeneration as compared with normal.

Comparison of ultraviolet photographs of normal and regenerating liver (fig. 4) showed morphologic changes, such as increased fat vacuolation of cytoplasm and increased size of nuclei and nucleoli. Furthermore, these photographs showed an augmented cytoplasmic absorption of ultraviolet rays, especially noticeable adjacent to the nuclear membrane.

This presumptive evidence of increased nucleic acid in regeneration is confirmed by the quantitative cytochemical and macrochemical data to be mentioned. Although these sections are unstained and relatively unfixed by the freezing-drying process employed, they provide a picture in ultraviolet absorption similar to that seen with stained fixed sections in visible light. Comparison of ultraviolet photographs of such frozen-dried tissue and tissues fixed in Carnoy or in Stieve fluid showed them to be quite similar.

The results of densitometric measurements of ultraviolet photographs of frozen-dried normal and regenerating cells are given in table 3. The values for normal tissue included measurements from three different specimens; those for 36 hours regeneration were from 2 rat livers, and all others from 1 liver. Sometimes more than one nucleolus was measured in the same nucleus. The direct comparison of measurements of extinction coefficients of cells in different sections is difficult because

TABLE 3.—*Mean Ratios of Absorption Measurements of Wavelengths of 257 to 275 Millimicrons and of Protein and Nucleic Acid in Nucleoli and Cytoplasm of Regenerating Liver Cells*

Period of Regeneration, Hr.	Cells	Nucleolus		Cytoplasm	
		E257	P	E257	P
		E275	NA	E275	NA
0	23	1.16	34	1.09	45
12	4	1.12	40	1.07	43
24	39	1.18	32	1.11	42
30	12	1.21	27	1.11	42
36	9	1.30	18	1.13	38
42	4	1.40	13	1.27	20

slight variation of section thickness can produce considerable changes in ultraviolet absorption. Therefore, when one is not comparing parts of the same section, it is often better to relate the absorption at 257 millimicrons, which is markedly affected by nucleic acids, to that at 275 millimicrons, which, predominantly produced by proteins, is much less readily affected in changes of the concentration of protein or nucleic acid. The use of ratios, furthermore, reduces errors from the inability to correct for light scattering and dispersion in the photographed tissues. The mean ratios of extinction coefficients of 257 to 275 millimicrons shown in table 3 indicate that there is an increase during regeneration in the total nucleic acid of the nucleoli and of the juxtannuclear cytoplasm. Statistical analysis showed that this increase over normal is significant at 42 hours for both nucleolus and cytoplasm and at 36 hours for the nucleolus. The ratios of protein (P) to nucleic acid (NA) are taken from data compiled from absorption measurements of a standard protein by Caspersson and Santesson.¹⁴

14. Caspersson and Santesson,^{3d} p. 38.

To check macrochemically the ultraviolet cytochemical measurements, Prof. E. Hammarsten¹⁵ made determinations of total phosphorus and of percentage ribonucleotides of all nucleotides on several control and regenerating liver specimens from these same animals. The

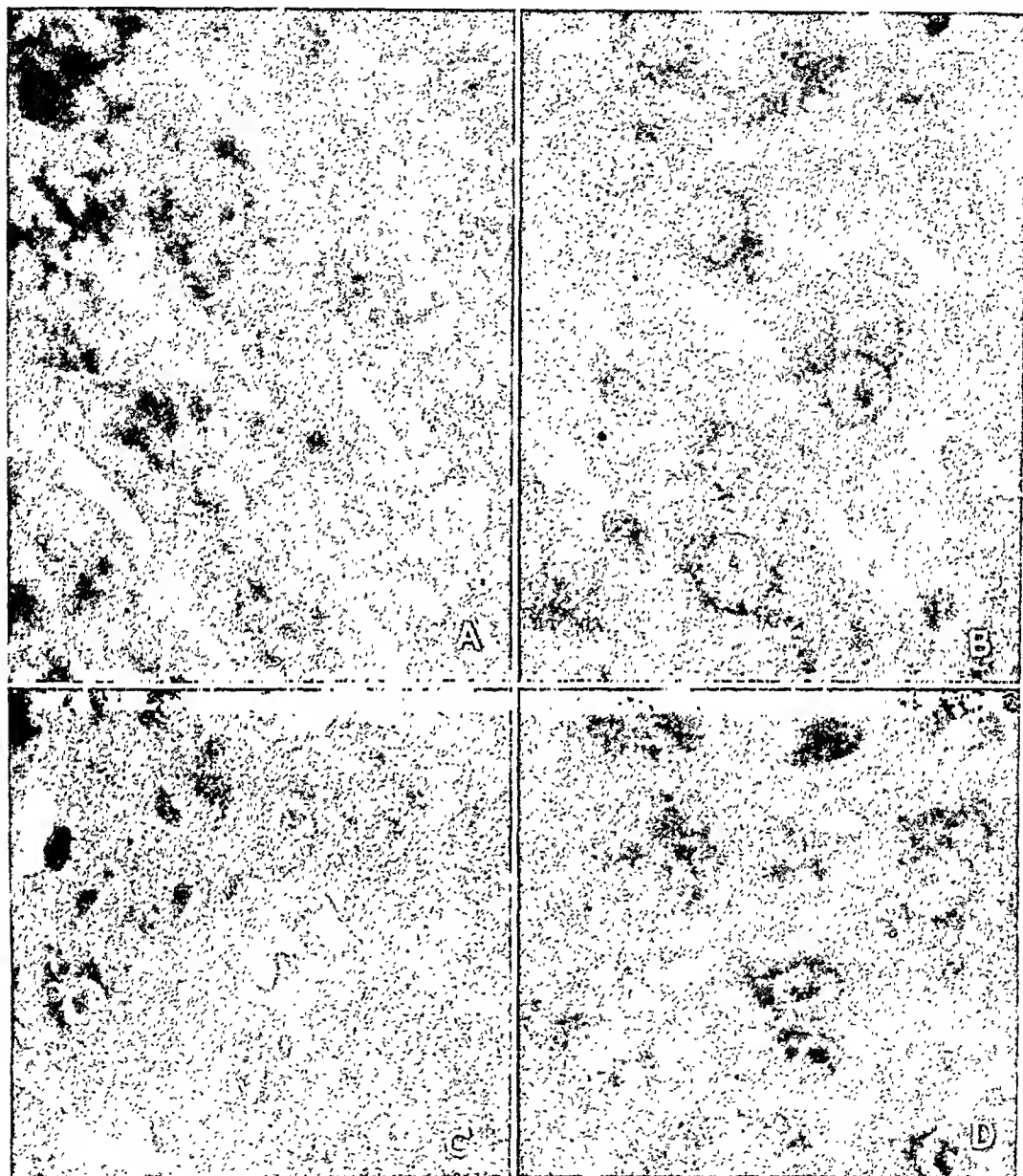


Fig.4.—Sections of unstained rat liver all photographed at 257 millimicrons wavelength; $\times 1,000$.

A, high juxtannuclear absorption and vacuolated cytoplasm seen in this area at 24 hours' regeneration; tissue fixed in Stieve solution.

B, normal liver; frozen, dried tissue.

C, 24 hours' regeneration; frozen, dried tissue.

D, 36 hours' regeneration; frozen, dried tissue.

15. Hammarsten, E.: Personal communication of results, with methods to be published.

results in table 4 indicate an increase in total nucleic acid as well as in the ribose fractions during liver regeneration. With the technic employed, the low nucleotides were less than 10 per cent of the total nucleotides.

COMMENT

A comparison of the morphologic and cytochemical measurements indicates interesting relationships. Within the first six hours of regeneration the cells showed a significant increase in the volumes of cytoplasm, nucleus and nucleolus and a decrease in vascular space. The increased visible cytoplasmic lipids suggest altered cellular metabolism. The earliest swelling of the hepatic cells may be associated with increase of lipids and imbibition of fluids rather than being primarily accounted for by increased protein synthesis. Although the volume of the total liver, as well as that of the cells, increased considerably, relatively few cells were dividing during the first day. By 24 hours the mean cyto-

TABLE 4.—*Macrochemical Determination of Percentage of Total Phosphorus and of Percentage of All Nucleotides That Were Ribose Type in Regenerating Liver Cells (Hammarsten¹⁵)*

Stage of Regeneration Represented in Specimen	Total Phosphorus, Percentage	Ribose, Percentage
Control.....	0.43	64
12 hr.....	0.44	70
24 hr.....	0.53	70
42 hr.....	0.56	70

plasmic, nuclear and nucleolar volumes had increased 2.4, 2.2 and 4.1 times, respectively; yet the absorption coefficient measurements suggest no appreciable change in percentage composition of nucleic acid and protein in either nucleolus or cytoplasm. The incidence of cell division increased greatly at 30 hours and was accompanied, as might be expected, by some decrease in mean volume of cytoplasm, nucleus and nucleolus. Marshak and Byron¹⁶ found that the percentage of cells in mitosis increased from 0.03 to 0.37, 0.47 and 0.27 on the first, second and third day of regeneration. Brues and Marble¹⁷ reported an increase from less than 0.001 per cent mitoses to 2.1 per cent at 24 hours with a decline toward normal during the next two days. Thus, all cells show an initial tremendous increase of their constituents, but after they start dividing, their mean volume decreases. With the augmented mitotic activity and associated production of cellular constituents there is good cytochemical evidence of an increase in the concentration of nucleic acids in the cytoplasm near the nuclear membrane. Although

16. Marshak, A., and Byron, R. L.: *Proc. Soc. Exper. Biol. & Med.* **59**:200, 1945.

17. Brues, A. M., and Marble, B. B.: *J. Exper. Méd.* **65**:15, 1937.

the number of cells measured is relatively small at some stages of regeneration, nevertheless the results show a consistent change.

Davidson and Waymouth¹⁸ made macrochemical determinations on nucleic acid in liver under several conditions. In regenerating liver they found that the total nucleoprotein phosphorus was not appreciably altered, the acid-soluble nucleotide concentration was raised and the ratio of ribonucleic acid phosphorus to desoxyribonucleic acid phosphorus was unaltered.

In regenerating as compared with normal rat liver Brues, Drury and Brues^{4a} found 19.5 per cent less percentage nitrogen (wet weight) the first day, 18.2 per cent less the second and 7.1 per cent less the third. Although the liver was restored at a somewhat faster rate in this experiment than in theirs, it seems probable that there is increased tissue fluid with little new cellular protein formed in the first day, with considerably more in the second and third days. Measurements of nucleic acids beyond the second day of regeneration might have shown a greater increase in their concentration.

Brues, Tracy and Cohn¹⁹ studied the turnover of radiophosphorus in regenerating and normal liver. Both types of nucleic acid had a greatly increased activity in regeneration, which was attributed to synthesis and turnover.

The interpretation of the chemical changes in the nucleoli is not simple. Although the thickness of the cytoplasm should be similar for comparing photometric measurements of sections of different tissues, in comparing nucleoli one should consider changes in their diameters or volumes. Nucleolar diameters were measured on the photographic plates for those nucleoli on which densitometric determinations were made. Although the computed mean volume of nucleoli measured by this method showed more than twice the normal size at 24 hours, the extinction coefficients for 257 and 275 millimicrons were considerably decreased. Furthermore, the mean extinction coefficients for both wavelengths increased consistently during the subsequent phases of regeneration when mean nucleolar size was decreasing. The evidence from the respective extinction coefficients and their ratios to each other suggests that nucleoli of dividing regenerating cells have a higher concentration of nucleoproteins and especially of the ribose nucleic acid component, which is the principal type found in the nucleolus. The methods employed would, however, measure both the constituents of the nucleolus as well as the overlying nuclear chromatin, which is fortunately scant by comparison with the nucleolar material. Larger nucleoli would more frequently be divided in cutting sections, so that less of

18. Davidson, J. N., and Waymouth, C.: *Biochem. J.* **38**:379, 1944.

19. Brues, A.; Tracy, M. M., and Cohn, W. E. P.: *J. Biol. Chem.* **155**:619, 1944.

their total substance would be measured. These sources of error were reduced by utilizing comparative data on numerous cells. Additional experiments on the chemical composition of the nucleolus would be desirable.

Although specimens from each liver were usually fixed and prepared by seven methods, which gave somewhat similar results, the freezing-drying technic was the most generally satisfactory for cytochemical work. It produced less artefact from scattering and dispersion of light by the protoplasmic matrix of cells.

Fortunately, this experiment permits one readily to relate the cytochemical and morphologic data for the regenerating liver to those for the normal liver from the same animal. The expression of results in such relative terms may be much more accurate than that based on the use of more highly quantitative units.

With polyploidy of the liver cells one might expect the increases of chromosomal content to be accompanied by comparable increases of nuclear volume to give groups of nuclei with mean values having ratios of 1:2:4:8:16.

Bieselev, Poyner and Painter²⁰ and Sulkin⁴⁰ have computed the volumes of liver cell nuclei by different methods and reported groupings of different-sized nuclei. The first group of investigators made measurements usually on less than 100 nuclei per specimen and found classes with ratios of 1:2.3:4.1. Their class 1 contained a variety of types of cells, including endothelial and blood cells. Sulkin measured 200 nuclei on each of 6 normal and regenerating rat livers and obtained classes with ratios of 1:2.0:4.2. After 28 days of restoration the liver cell nuclei still fell into comparable classes with the same maximum for each mode, but with more of the larger size nuclei.

Beams and King²¹ have discussed the relationship of binucleate cells and polyploidy in restoring liver at three days. They found about 20 per cent binucleate cells in both normal and regenerating livers. Plotting of nuclear diameters against their number showed a bimodal curve. The nuclear volumes of the first mode in the second had a ratio of 1:2, with more than twice as many nuclei in the first group of uninuclear cells. Binucleate cells had a similar bimodal curve, with more nuclei in the group with the larger volume. In restoring liver the second mode of the bimodal curves was more scattered, with variable numbers of larger nuclei. For the uninuclear cells the volume of each mode was increased nearly a fourth and the ratio of the first and second modes was about 1:1.7.

20. Bieselev, J. J.; Poyner, H., and Painter, T. S.: Nuclear Phenomena in Mouse Cancers, Publication 4243, University of Texas, 1942.

21. Beams, H. W., and King, R. L.: Anat. Rec. 83:281, 1942.

Analysis of nuclear volumes on 100 cells in each of the 13 normal livers in this experiment showed that they tended to fall into two distinct classes with a ratio of 1:1.75. There was suggestive evidence of the presence of other higher classes, but the number of measurements was inadequate to establish them definitely. There was reasonably close agreement between the classes in different normal livers, the ratios varying from 1:1.6 to 1:2.1. In regenerating livers such ratios were obtained only at stages under 24 hours. At intervals of 24 to 48 hours there was usually loss of significant modal distribution, even though 500 nuclei were measured on several specimens. This absence of distinct classes may be attributed to the mixture of (1) cells with enlarged nuclei which have not divided with (2) recently divided cells. It is evident that normal liver cell nuclei tend to form several groups related to their chromosome content and that the volumetric relationship of the groups is altered or lost in certain stages of restoration of the liver as it is in many cancers. It is not clear why this study and those of Sulkin^{4e} and Bieseke, Poyner and Painter²⁰ found more nuclei in the second class than in the first whereas Beams and King²¹ found more in the first in mononuclear cells. The percentage of binucleate cells (20) reported by Beams and King is inadequate to explain this difference.

These experiments, in conjunction with other observations, including those on changes in the nucleoli of the liver cells of rats restricted to low and high protein diets and those made during production of hepatomas with para-dimethylaminoazobenzene,²² add material support to the evidence for an important relationship between the nucleolus and the cytoplasm in the formation of nucleic acids and proteins. It seems desirable to defer the more theoretic considerations of the relationships of these morphologic and chemical changes in the nucleolus and the cytoplasm until further experimental data are presented.

SUMMARY AND CONCLUSIONS

The changes in cellular structure and nucleic acid concentration were correlated during the first two days of regeneration of rat liver. Within the first six hours of regeneration there was an increase in the visible cytoplasmic lipids and in the volume of the cytoplasm, nucleus and nucleolus with a decrease in the sinusoidal space. By 18 to 24 hours the mean volumes of the cytoplasm, the nucleus and the nucleolus had increased 2.6, 2.2 and 4.1 times, respectively. Following an increase in cell division at 24 to 30 hours, there was a decrease in the mean volume of each of these cellular constituents. At 48 hours' regeneration the mean nuclear and cytoplasmic volumes were still

22. Stowell, R. E.: Cancer, to be published.

considerably above normal, while the mean nucleolar volume was less than normal. In normal livers the nuclear volumes constituted two main groups with mean ratios of approximately 1 to 2. This evidence of normal polyploidy tended to disappear in the second day of regeneration.

Ultraviolet cytochemical observations indicated little change in nucleic acid concentration during the first 24 hours, but during the second day the synthesis associated with the rapid cell division was accompanied by an increase of nucleic acid concentration in the cytoplasm adjacent to the nucleus and also in the nucleolus. The results indicate that the morphologic aspects and the chemical composition of the nucleolus and of the cytoplasm are greatly altered in different phases of growth in regenerating hepatic cells.

University of Kansas.

MITOTIC ACTIVITY IN THE AORTIC LESIONS OF EXPERIMENTAL CHOLESTEROL ATHEROSCLEROSIS OF RABBITS

GARDNER C. McMILLAN, M.D., Ph.D.*

AND

G. LYMAN DUFF, M.D., Ph.D.

MONTREAL, CANADA

THE relatively frequent occurrence of mitotic configurations in the globular foam cells and in the stellate fibroblastic cells of the aortic lesions induced by cholesterol feeding in rabbits appears to have escaped the attention of those interested in the morphologic aspects of experimental cholesterol atherosclerosis. The reviews and reports of such workers as Anitschkow,¹ Leary,² Duff³ and Hueper⁴ do not mention mitotic activity. There is general agreement that the fibroblastic elements of the atherosclerotic lesions proliferate, and Hueper⁴ stated that vascular endothelial cells multiply and are transformed into globular foam cells. The concept that some, or all, of the lipophages contained in the arterial lesions of experimental cholesterol atherosclerosis arise *in situ* by mitotic division was considered theoretically by Duff,³ but without the support of morphologic evidence of mitotic activity.

It is our purpose in this report to present evidence that mitotic figures are not uncommon in the cellular components of the intimal lesions of experimental cholesterol atherosclerosis of the aorta.

MATERIALS AND METHODS

The mitoses reported here were found in the atherosclerotic lesions of the aortas of 10 rabbits fed cholesterol as a 5 per cent solution in warm corn oil in a daily dose of about 0.75 Gm. The period of feeding varied from seventy-six to ninety days, and the total dose of cholesterol administered to each animal was from 46 to 65 Gm. On completion of the feeding experiment the animals were killed by air embolism, autopsies made and the opened aortas fixed in 10 to 15 per cent concentration of

* Medical Research Fellow of the National Research Council, Canada.

From the Department of Pathology, Pathological Institute, McGill University.

This work was assisted by grants-in-aid from the National Research Council, Canada.

1. Anitschkow, N., in Cowdry, E. V.: *Arteriosclerosis: A Survey of the Problem*, New York, The Macmillan Company, 1933, chap. 10.

2. Leary, T.: *Arch. Path.* **17**:453, 1934.

3. Duff, G. L.: *Arch. Path.* **20**:81 and 259, 1935.

4. Hueper, W. C.: *Arch. Path.* **38**:162, 245 and 350, 1944; **39**:51, 117 and 187, 1945.

formaldehyde solution U. S. P. in saline solution. After fixation the aorta was rolled into a coil and embedded in paraffin so that a single microscopic section could be made to include the entire length of the organ. Sections 6 microns in thickness were stained with Mallory's phosphotungstic acid-hematoxylin, hematoxylin and eosin or hematein, phloxine and Spanish saffron. The time from death until the aorta was placed in fixative varied from about twenty to ninety minutes. All animals suffered from a relatively severe degree of atherosclerosis of the aorta with extensive confluent intimal plaques measuring up to about one half the thickness of the underlying media.

The criteria of mitotic configuration were those commonly accepted, including the proviso that the phase of mitosis should be clearly recognizable. If a figure was recognized as mitotic but, for technical reasons, the phase of activity was not clearly indicated, then the figure was classified as one of undetermined phase. Configurations that were merely suggestive of mitosis or that represented frank necrobiotic phenomena were excluded.

Summary of Data on Mitotic Activity in Foam Cells and Fibroblastic Cells in Experimental Cholesterol Atherosclerosis of the Aorta

Rabbit	Distribution of Mitoses by Phase				Number in Fibro- blasts	Number in Foam Cells	Total Mitotic Mitoses Figures of Recog- Undeter- nized Phase mined Phase		Total
	Pro- phase	Meta- phase	Ana- phase	Telo- phase					
1.....	1	2	0	0	0	3	3	4	7
2.....	3	4	0	0	2	5	7	3	10
3.....	0	1	0	1	0	2	2	4	6
4.....	1	1	0	0	0	2	2	2	4
5.....	0	1	0	0	1	0	1	0	1
6.....	1	8	1	1	3	8	11	7	18
7.....	0	0	0	1	0	1	1	1	2
8.....	0	3	0	1	0	4	4	0	4
9.....	1	4	0	0	0	5	5	4	9
10.....	0	4	4	1	2	7	9	7	16
Total.....	7	28	5	5	8	37	45	32	77

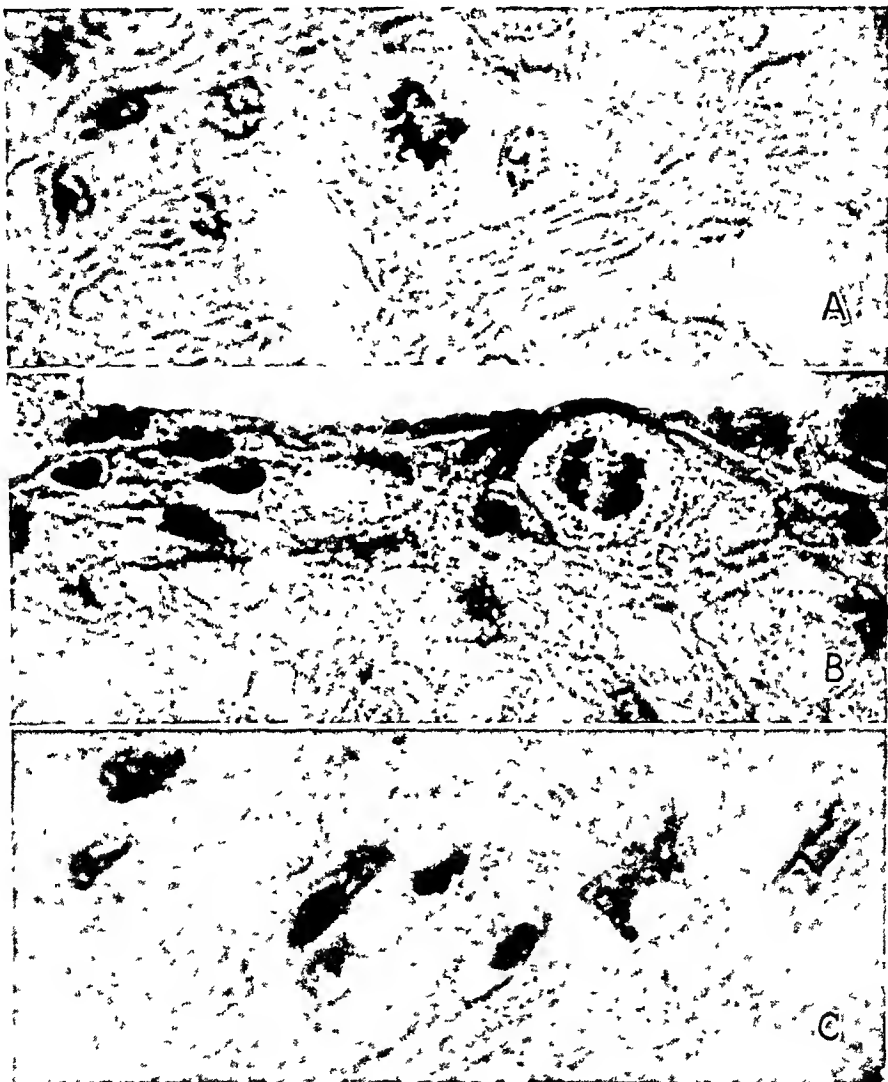
OBSERVATIONS

Among the 10 aortas examined, 6 presented lesions that proved on microscopic examination to consist almost entirely of foam cells. The remaining 4 aortas also presented varying degrees of intimal fibrosis.

A summary of the incidence and phase of the mitotic figures encountered in a single section of the entire length of the aorta of each animal is given in the table. Photomicrographs of some of the different phases of mitotic division in lipophages are shown in the figure.

The table shows that 45 mitotic figures of identifiable phase were observed in the 10 aortas. Of these, 37 were in globular foam cells, and 8 were in fibroblastic cells. Inasmuch as the mitotic figures recorded were encountered in a single section of the entire length of the aorta of each animal, the average number of mitoses seen in the lipophages of each section was 3.7, or about 1 such figure in every 4 cm. of length of the section. In the 4 aortas in which intimal fibrosis also occurred, 8 figures were seen in fibroblastic cells, or about 1 figure in each 7 or 8 cm. of length of the section.

In addition to these 45 mitoses, 32 mitotic figures of uncertain phase of division were observed. If these configurations are also taken into account, an average of 1 mitotic figure was seen in every 2 cm. of length of the sections of aorta 6 microns in thickness. It will be noted that the number of figures seen in each section varied considerably, the least being 1 and the greatest being 18.



Photomicrographs of mitotic figures in foam cells in aortic intimal lesions of experimental cholesterol atherosclerosis: *A*, a mitotic figure in metaphase.

B, a mitotic figure in anaphase.

C, a mitotic figure in telophase.

Mitotic figures were observed in all layers of the atherosclerotic lesions from the most superficial to the deepest, but it was our impression that they were somewhat more frequent in the more superficial cells. Mitotic figures were not associated with signs of degeneration or necro-

sis of adjacent cells. Indeed, in the single aorta in which there was patchy necrosis in the deeper layers of the intimal lesions, mitoses were not observed in relation to these necrotic areas. In addition to the evidence of cellular division, there was evidence of nuclear division alone, demonstrated by the presence of numerous large binucleate foam cells, in the deeper layers of the intimal lesions. In the 10 sections examined, mitotic figures were not observed in the lining endothelial cells.

COMMENT

Before concluding that the mitotic figures observed were truly indicative of cellular division, the possibility was considered that these mitoses might represent necrobiotic or postmortem phenomena. This possibility was excluded by the observation that almost one quarter of the mitotic figures were in typical anaphase or telophase, indicating true mitotic activity, since mitonecrotic division seldom progresses beyond metaphase. Moreover, mitotic figures were not observed to be associated with areas of degeneration or necrosis. The observation of large, well preserved binucleate foam cells in the deeper layers of the atherosclerotic lesions may be interpreted as further evidence that nuclear division is carried to completion in living cells, even though in these particular cells cytoplasmic division apparently failed to occur.

The data presented are inadequate to permit a detailed analysis of the factors concerned in the production of mitotic division in the cellular components of the lesions of experimental cholesterol atherosclerosis of the aorta. Nevertheless, the occurrence and frequency of mitotic division in these cells are evidence that a considerable proportion, if not all, of them arise by local proliferation of preexisting cells of the same types. Accordingly, there remains no compelling reason to assume that the increase in numbers of lipophages in the developing intimal lesions is dependent on a continuous process of migration of such cells into the intima either from the lumen of the artery or from the medial direction. Neither do our observations lend support to the concept that the cellular growth of these lesions depends on repeated mitotic divisions of the lining endothelial cells. Regardless of what theory is proposed relative to the derivation and original source of the foam cells and fibroblasts in the lesions of experimental cholesterol atherosclerosis, it is apparent that a considerable proportion of them arise *in situ* by mitotic division.

SUMMARY

The observed frequent occurrence of mitotic figures in the foam cells and fibroblasts of the aortic intimal lesions of experimental cholesterol atherosclerosis of the rabbit is interpreted as evidence that a considerable proportion, if not all, of these cells arise *in situ* by mitotic division.

Case Reports

DIFFUSE PLASMA CELL MYELOMATOSIS

E. STARK, M.D., and E. L. AMIDON, M.D.
BURLINGTON, VT.

THE CONCEPT that plasma cell myeloma is not primarily an osseous lesion but is a diffuse neoplastic disorder of the hemopoietic system has been steadily gaining recognition.¹

There are numerous cases on record in which one or more of the viscera are involved by myelomatous masses. Diffuse plasma cell involvement of the viscera is infrequent and is usually associated with the appearance of plasma cells in the blood stream. These cases are segregated under the heading of plasma cell leukemia.¹ That diffuse plasma cell myelomatosis may occur without a concomitant leukemic blood picture is shown in the case reports of Lowenhaupt.²

Because of the rarity of completely studied cases, an instance of diffuse plasma cell myelomatosis is presented.

REPORT OF A CASE

A 51 year old salesman was admitted to the hospital complaining of pain in the right lateral aspect of his chest. Ten months previously he had profuse and continued rectal bleeding, for which he was given a blood transfusion. Two months previously he first noted loss of appetite and loss of weight, and about this time he noted jaundice, and his liver was found to be enlarged. On admission he stated that he had lost a total of 13.5 Kg. (30 pounds). He had had recent attacks of epistaxis. He was not in the habit of taking alcoholic beverages. Physical examination revealed an obese, well developed, jaundiced man, breathing with difficulty because of thoracic pain. His blood pressure was 98 systolic and 50 diastolic. The heart sounds were rapid, with no murmurs. In the right lower region of the chest anteriorly, dullness, diminished breath sounds and coarse snapping rales were heard. The abdomen was enlarged, with visible veins and ecchymoses. The edge of the liver extended to 10 cm. below the costal margin. He had a left-sided hydrocele. The rectal examination gave negative results.

The significant laboratory data were as follows: red cell count, 1,200,000; hemoglobin content, 26 per cent; white blood cell count, 5,000, with 56 per cent polymorphonuclears, 40 per cent lymphocytes, 4 per cent eosinophils and occasional nucleated red cells. After the autopsy, reexamination of his blood smears revealed 1 to 2 per cent plasma cells. The urine revealed small amounts of albumin but no Bence Jones protein. On admission the icterus index was 50 units; terminally it was 100 units. Serum albumin was 5.3 mg., globulin 1.5 mg. and nonprotein nitrogen 55 to 76 mg. per hundred cubic centimeters. Prothrombin activity was 50 per cent of normal, decreasing to 20 per cent of normal. Aspirated sternal

From the Departments of Pathology and Medicine, University of Vermont College of Medicine.

1. Lubarsch, O.: *Virchows Arch. f. path. Anat.*: **184**:213, 1906. Jackson, H., Jr.; Parker, F., Jr., and Bethea, J. M.: *Am. J. M. Sc.* **181**:169, 1931. Piney, A., and Riach, J. S.: *Folia haemat.* **46**:37, 1931. Moss, W. T., and Ackerman, L. V.: *Blood*. **1**:396, 1946.

2. Lowenhaupt, E.: *Am. J. Path.* **21**:171, 1945.

marrow revealed a preponderance of plasma cells, which were in various stages of development, with many irregular forms (fig. 1).

Roentgenograms revealed widespread small osteolytic lesions of the ribs, the scapulas and to a lesser extent of the spinal column and the pelvis. A barium sulfate enema and a gastrointestinal series showed no abnormality.

The patient's course was steadily downhill, with increasing weakness, deepening jaundice and terminally many petechial hemorrhages. His temperature remained at about 101 F. His spleen became palpable after the second week of hospitalization. He died on the twenty-eighth hospital day.

Autopsy.—The body was that of a white man and weighed 84 Kg. (185 pounds) and measured 180 cm. The skin and the scleras were deeply icteric. Numerous petechial hemorrhages were noted on the arms, the thighs and the legs. The super-

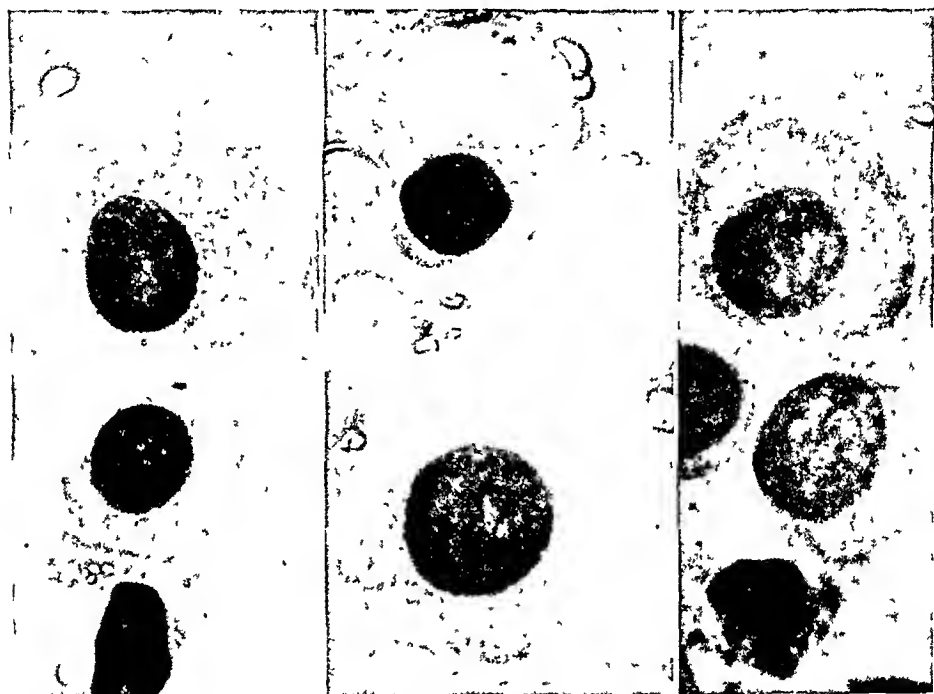


Fig. 1.—Plasma cells in bone marrow smears, illustrating various stages of development and irregular forms; $\times 1,000$; Wright stain.

ficial lymph nodes were not enlarged. There was a recent biopsy incision over the tip of the right scapula.

The peritoneal cavity contained approximately 600 cc. of clear icteric fluid. The liver's edge extended 12 cm. below the costal margin in the right midclavicular line. Along the common bile duct there was a chain of lymph nodes which were firm and measured 2 to 3 cm. in diameter. The mesenteric and some of the retroperitoneal nodes were slightly enlarged.

The pleural cavity contained about 50 cc. of clear fluid on each side. There were fractures of the first, third and sixth ribs on the left side and the third and sixth ribs on the right side. At these sites palpable nodular enlargements with subpleural ecchymoses were found.

The mediastinal nodes were not enlarged.

The heart was not remarkable.

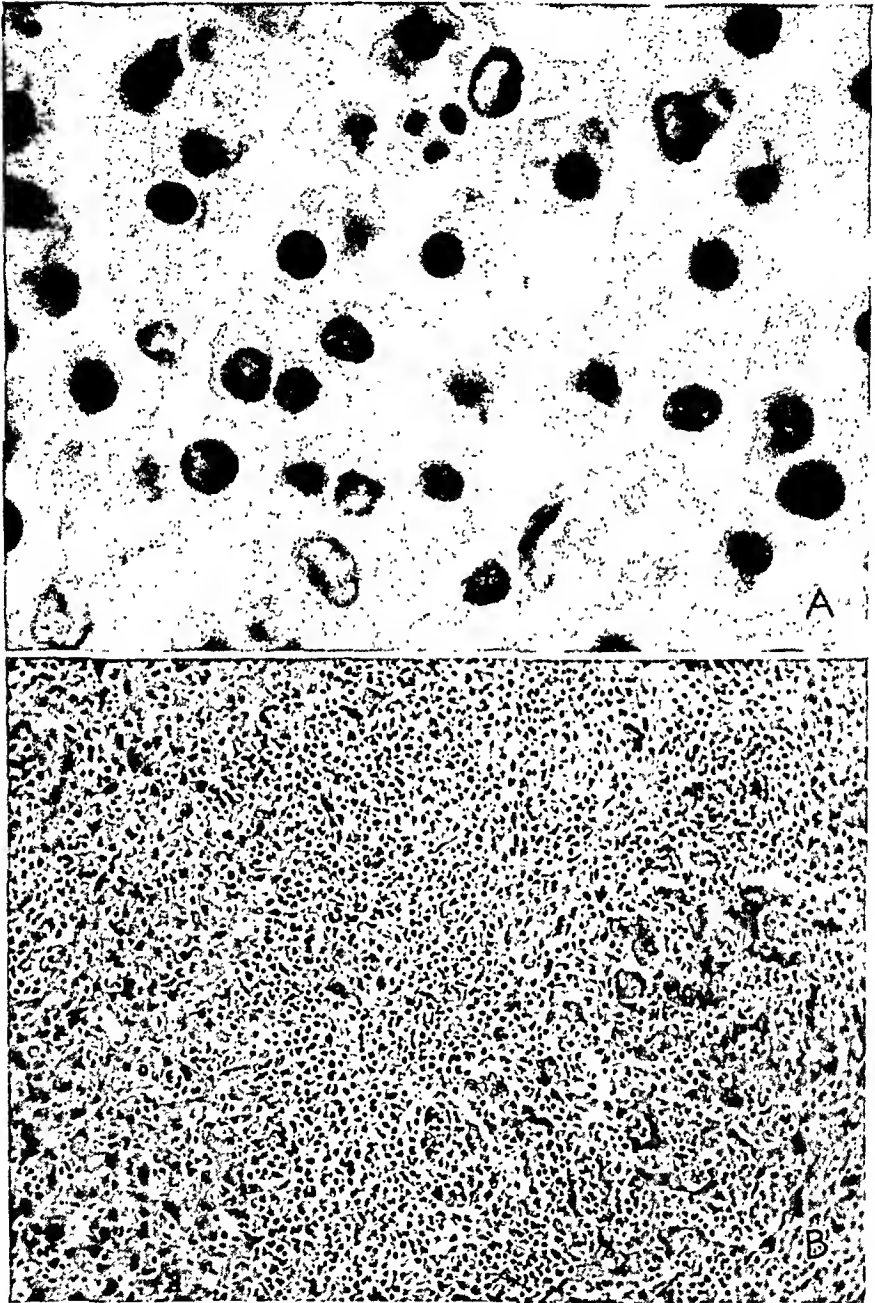


Fig. 2.—*A*, lymph node showing crowding of the sinusoids with plasma cells; $\times 1,000$; hematoxylin and eosin. *B*, liver showing extensive plasma cell infiltration of hepatic sinuses and periportal connective tissue; $\times 100$; hematoxylin and eosin.

The lungs showed severe congestion.

The spleen weighed 730 Gm., and the cut sections were homogeneously gray-red and moderately firm.

The liver weighed 3,400 Gm., the cut surfaces of which revealed the lobular structure accentuated by a pale gray markings. The biliary tract was patent.

The marrow of the vertebrae, the sternum and the ribs was replaced by diffuse grayish soft tissue. An incidental finding was an 8 cm. renal tumor which was typical of renal cell carcinoma ("hypernephroma") both grossly and microscopically.

No gross myelomatous nodules were found in any of the viscera.

Microscopic Examination.—The spleen revealed complete alteration of structure with absence of follicles and diffuse plasma cell infiltration in various stages of development. While many of the larger cells had nuclear and cytoplasmic characteristics akin to those of reticulum cells, others had the distinctive features of well differentiated plasma cells.

The lymph nodes showed even more complete plasma cell replacement of all elements (fig. 2 A).

In the liver, cellular infiltration was most striking, being diffuse throughout the parenchyma and especially dense in the peripheral zones of the lobules (fig. 2 B). This was associated with severe retrogressive changes of the hepatic cords. Bile casts were present in many of the ductules.

The lungs showed severe interstitial pneumonia, in connection with which numerous plasma cells were found together with other mononuclear cells.

The kidneys, aside from the carcinoma, showed normal glomeruli, but severe tubular degeneration was evident, with fibrosis of the interstitial tissue and occasional focal collection of plasma cells.

The marrow of the sternum, the ribs and the vertebrae revealed diffuse plasmacytic infiltration. These cells were found freely intermingled with the other marrow elements rather than as compact "plasmacytomas."

COMMENT

The anatomic distribution of plasma cells illustrated in the case described differs in no essential feature from that observed in other forms of leukemia with the exception that there was no invasion of the blood stream. For this reason the term "plasma cell myelosis" or "plasma cell myelomatosis" may be applied in such cases. To refer to the cell type as "myeloma cell," as has been the tendency in the recent literature,³ does not seem any more reasonable than to call the abnormal lymphoid cells appearing in lymphatic leukemia by a special name. The cells of myelomatosis can usually be identified as belonging to the plasma cell series, and various developmental stages may be observed (fig. 1). Admittedly, many bizarre forms may occur in varying proportion, just as in other forms of leukosis. The "typical" cart wheel nucleus of the plasma cell as seen in tissue sections is practically never seen in marrow smears and is probably due to fixation.

SUMMARY

A case is presented in which at autopsy a white man was revealed to have diffuse plasma cell infiltration of the viscera of a type usually seen in plasma cell leukemia, but in this instance without a significant number of plasma cells in the blood stream.

3. Winthrobe, M. M.: Clinical Hematology, Philadelphia, Lea & Febiger, 1946.

ADENOMA OF THE PAROTID GLAND

JOHN T. GODWIN, M.D.
NEW YORK

and

S. H. COLVIN Jr., M.D.
NEW ORLEANS

ACKERMAN,¹ in 1943, reviewed the literature, tabulated 7 cases which he felt were authentic and added a new case. Salivary gland adenoma is also termed oncocytoma, since it supposedly arises from oncocytes. Stout² recently quoted the work of Hamperl, who originated the term "oncocyte" and listed the various organs in which this type of cell is found. Oncocytes of the salivary glands reportedly take origin from the tubules and terminal secreting portions of seemingly normal tissue.³ Ackerman and Regato⁴ stated that oncocytoma is an adenoma and that probably in many instances it arises from duct epithelium. Gruenfeld,⁵ in reporting a case of oncocytoma, stated that the histologic picture suggested an origin from the duct system. Schutz,⁶ in quoting Lambret, mentioned that salivary adenoma is of two types and originates from either duct epithelium or acinous epithelium. Gruenfeld⁵ stated that Huckel and Franssen described parathyroid-like new growths of the parotid gland which they believed to be adenoma developing from the parenchymal cells.

The case to be reported is one of adenoma of the parotid gland, but it differs from the reported cases in that the tumor apparently arose from acinous epithelium.

REPORT OF CASE

H. S., a 38 year old white woman, complained of swelling of the left side of the face, which was first noticed two years previously. The mass steadily increased in size. At no time was it painful, although a stinging sensation occasionally occurred, lasting for a few seconds. The lesion was soft and small in the morning but by late afternoon was large and firm. Located at the angle of the left mandible was a well defined mass about 2.5 cm. in diameter which was not tender and not attached to the overlying skin.

At operation a well encapsulated soft tumor was found within the parotid parenchyma. It was completely removed with a minimal amount of the surrounding

From the Department of Pathology, Touro Infirmary.

1. Ackerman, L. V.: *Arch. Path.* **36**:508, 1943.
2. Stout, A. P.: *Arch. Path.* **35**:803, 1943.
3. Gruenfeld, G. E., and Jorstad, L. H.: *Am. J. Cancer* **26**:571, 1936.
4. Ackerman, L. V., and del Regato, J. A.: *Cancer Diagnosis, Treatment and Prognosis*, St. Louis, C. V. Mosby Company, 1947.
5. Schutz, C. B.: *Am. J. Path.* **2**:153, 1926.

parotid gland. In removal the capsule was ruptured, with liberation of brown mucoid material. The specimen was placed in 4 per cent formaldehyde solution, sectioned and stained with hematoxylin and eosin, Mayer's mucicarmine, Mallory's aniline blue, Masson's trichrome stain and sudan IV.

Grossly the tumor measured about 4 cm in diameter. The tissue was soft and appeared homogeneously pinkish gray. The capsule was complete except for the point of surgical rupture.

Microscopic sections showed the tumor to be completely encapsulated by fibrous tissue and surrounded by small areas of normal-appearing salivary gland. There was a single lymph node external to the capsule. In the capsule were a few focal collections of lymphocytes. The cells composing the neoplasm were arranged in sheets with occasional acinus formation, which was most noticeable at the periphery. The cells were better stained in the peripheral portions of the tumor, where they showed a well defined cell wall and an eccentric nucleus. The cytoplasm varied

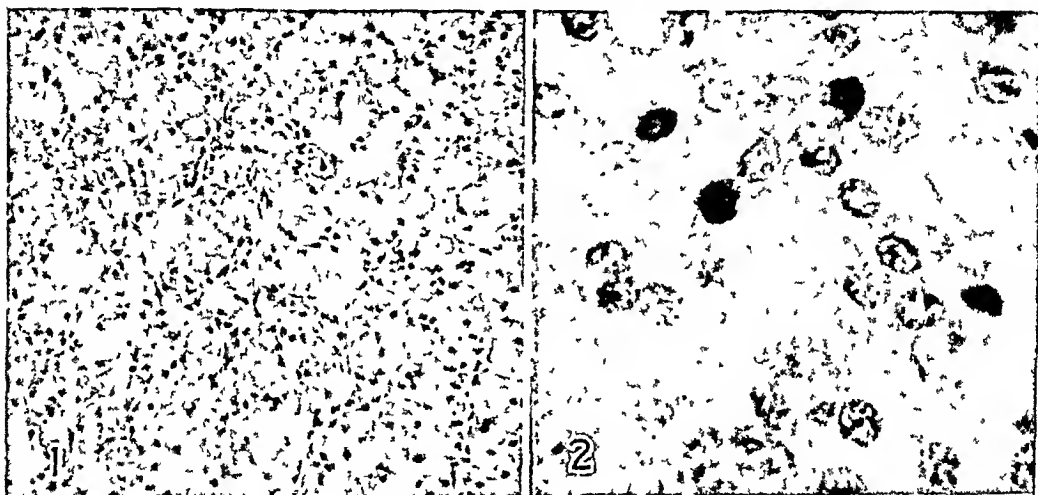


Fig. 1.—Adenoma of a parotid gland; hematoxylin and eosin; $\times 90$

Fig. 2.—Section of the tumor, showing vacuolation and the nuclear and cytoplasmic structure, hematoxylin and eosin stain; $\times 450$

from finely granular to vacuolated. The granular cells composed the major portion of the tissue, and their cytoplasm took a pale eosinophilic stain. The nuclei were predominantly round or oval, light staining and finely stippled. A few were small and stained dark blue. Between the cells were many vacuoles which resembled the sites of secreted material. Many of the vacuolated cells appeared to have ruptured with extravasation of their material. In some areas there were wide zones of vacuolated homogeneous light pink-staining material resembling colloid. No mitotic activity was observed. The vascularity was not pronounced, and the stroma was sparse, there being only occasional strands of fibrous tissue scattered through the tumor. No ductal structures were demonstrated. An occasional small focal accumulation of lymphocytes was present. The mucicarmine stain gave negative results, and sudan IV revealed finely divided interstitial fatty particles. The vacuolated areas revealed nothing with sudan IV. Masson's stain did not demonstrate eosinophilic granules. Mallory's stain revealed fine dark blue cytoplasmic granules, which were similar to those seen in the acinous cells of normal glands with control stains.

COMMENT

In the few reported cases¹ salivary gland adenoma occurred more commonly in women around the sixth decade and had a duration of a few months to several years. It attained a size of several centimeters, was usually firm, gray to brownish red and not attached to the skin. It had a characteristic microscopic picture.

In this case the tumor occurred in a 38 year old white woman. It was completely encapsulated. The tissue showed intercellular and intracellular vacuolation, intercellular colloid-like material and fine blue cytoplasmic granules. These three features suggest secretory activity and may explain the daily swelling of the tumor. No salivary ducts were found to suggest an origin from these elements, and none of the cells resembled the markedly eosinophilic cells termed oncocytes. The cells did not stain with sudan IV⁶ or with Masson's² trichrome stain as has been reported in regard to oncocytes. There has been no recurrence of the tumor after six months.

SUMMARY

Herein is reported an unusual case of adenoma of the parotid gland. It differs from the reported cases of adenoma (oncocytoma) in that the patient was younger, encapsulation of the growth was complete, acini were present and there was evidence of secretory activity. The individual cells resembled the acinous elements rather than the large granular eosinophilic cells seen in oncocytoma.

It is therefore believed that the reported adenoma arose from acinous epithelium in contradistinction to oncocytoma, which probably has a ductal origin.

6. Jaffé, R. H.: *Am. J. Cancer* 16:1415, 1932.

Books Received

EDUCATION FOR PROFESSIONAL RESPONSIBILITY: A REPORT OF THE PROCEEDINGS OF THE "INTER-PROFESSIONS CONFERENCE ON EDUCATION FOR PROFESSIONAL RESPONSIBILITY" HELD AT BUCK HILL FALLS, PENNSYLVANIA, APRIL 12, 13 AND 14, 1948. Pp. 207. Pittsburgh: Carnegie Press, 1948.

The conference was held to promote the interchange of experience among teachers of medicine, law, divinity, engineering and business. Three sessions were held, relating to: the objectives of professional teaching, 7 papers; the content and the method of professional education, 6 papers; social and humanistic aspects of professional education, 5 papers. The medical member of the planning committee is William M. Beckman, of Harvard University. The book will be of concern to educators in general and to teachers and deans in the disciplines mentioned. To medical teachers the following papers will be of special interest: "The Clinical Training of the Medical Student," by James H. Means, Jackson Professor of Clinical Medicine, Harvard Medical School; "A Social Worker Looks at Medical Education," by Eleanor E. Cockeril, professor of social case work, University of Pittsburgh; "The Physician as a Comprehensive Human Biologist," by John Romano, professor of psychiatry, University of Rochester School of Medicine and Dentistry.

OUTLINE OF HISTOLOGY. By Margaret M. Hoskins, Ph.D., and Gerrit Bevelander, Ph.D., New York University. Second edition. Pp. 113, with 56 illustrations. Price \$3.50. St. Louis: C. V. Mosby Company, 1948.

A brief survey of the elementary morphologic aspects of histology is presented in 286 pages, including interleaves for notes, and with one third of the text devoted to dental histology. There are 136 figures, mainly diagrams, including 2 colored plates on blood and several photographs of sections of teeth. There are no references to the literature. Except in the treatment of the nervous system, physiologic aspects are minimized. It is difficult to understand the statement, "In fresh blood, all leucocytes look much alike, and it is only the red cells which can be studied to advantage in the unstained drop of blood." Greater specificity might be preferred in the statement, "The spleen is interposed in the blood stream to remove *impurities* from the blood." (The italics are the reviewer's.) In general, cytologic details are omitted. In some sections the characteristics of the tissues are well summarized.

MEDICAL WRITING: THE TECHNIC AND THE ART. Morris Fishbein, M.D., editor of *The Journal of the American Medical Association*, with the assistance of Jewel F. Whelan, assistant to the editor. Second edition. Pp. 292, with 36 illustrations. Price \$4. Philadelphia: The Blakiston Company, 1948.

This guide to medical writing was first published as a small pamphlet in 1910. It is now a unique guide to the preparation of medical manuscripts of all kinds, especially, of course, those intended for any of the publications of the American Medical Association. Its use will help to advance the standards of medical writing.

INTRACRANIAL VASCULAR LESIONS IN LATE RHEUMATIC HEART DISEASE

JOHN DENST, M.D.

AND

KARL T. NEUBUERGER, M.D.

DENVER

ANATOMIC studies of the brain were long neglected in cases of chronic rheumatic heart disease. It is only during the last fifteen or twenty years that a number of papers dealing with this aspect of the rheumatic state have been published. Bodechtel¹ saw intimal proliferation in meningeal vessels in a case of chronic relapsing endocarditis. Von Sántha² studied a case of rheumatic chorea and found thrombosis with organization and occasionally also intimal proliferation and endothelial exfoliation. Productive endarteritic lesions were also present in the cortical capillaries and precapillaries and were sometimes associated with fibrinoid imbibition of their walls. The significance of such lesions in the genesis of chorea was more recently discussed by Buchanan, Walker and Case.³ Similar changes already had been observed by Winkelman and Eckel⁴ in 5 brains, which, however, came from patients with acute rheumatic fever. Kernohan, Woltman and Barnes⁵ described thrombotic and embolic lesions of small vessels in 23 cases, in most of which these lesions were associated with chronic rheumatic endocarditis. Lindsey⁶ described a case of ruptured rheumatic aneurysm of the posterior inferior cerebellar artery. Dublin⁷ found proliferative and thrombotic occlusions of vessels of various sizes in psychotic patients with chronic rheumatic heart disease. Recently Benda⁸ observed similar changes in the brains of mentally defective children. The most important papers dealing with the late intracranial sequelae of rheumatic fever are those of Bruetsch.⁹ He stated that obliterative

From the Department of Pathology, University of Colorado Medical Center.

1. Bodechtel, G.: *Ztschr. f. d. ges. Neurol. u. Psychiat.* **140**:657, 1932.
2. von Sántha, K.: *Virchows Arch. f. path. Anat.* **287**:405, 1932.
3. Buchanan, D. N.; Walker, A. E., and Case, T. J.: *J. Pediat.* **20**:555, 1942.
4. Winkelman, N. W., and Eckel, J. L.: *Arch. Neurol. & Psychiat.* **28**:844, 1932.
5. Kernohan, J. W.; Woltman, H. W., and Barnes, A. R.: *Arch. Neurol. & Psychiat.* **42**:789, 1939.
6. Lindsey, S.: *J. Nerv. & Ment. Dis.* **99**:717, 1944.
7. Dublin, W. B.: *Dis. Nerv. System* **2**:390, 1941.
8. Benda, C. E.: *J. Nerv. & Ment. Dis.* **106**:84, 1947.
9. Bruetsch, W. L.: *J. A. M. A.* **134**:450, 1947.

endarteritis of leptomeningeal vessels, with resulting gross and microscopic infarctions of the gray matter, is comparatively frequent. The relation of such lesions to psychiatric problems was considered by Bruetsch, and more recently, by Van der Horst¹⁰ and by Rojas and Vila.¹¹ The studies of Bruetsch were concerned exclusively with psychiatric patients. We felt that additional studies of the brain in unselected cases of chronic rheumatic heart disease were desirable. In a series of 688 consecutive autopsies there were 45 in which rheumatic heart disease was noted in patients older than 25 years, excluding those in whom it was complicated by subacute bacterial endocarditis. The brain was adequately examined in 14 of these cases, and in 9 it showed significant vascular changes.

Our series was not large enough to provide statistically reliable data on the incidence of cerebral lesions; however, we obtained the impression that in the majority of cases of chronic rheumatic heart disease vascular changes within the cranial cavity can be found readily by careful routine examination.

REPORT OF CASES

CASE 1.—A white woman of 34 years stated that she had had rheumatic heart disease and chorea at the age of 8. During the year prior to consultation she had epileptiform seizures, and for six months she had noted dysphagia, dysphasia, paresthesias of the arms, unequal pupils, and mental deterioration. She lost consciousness unexpectedly, pulmonary edema developed and she died.

The heart weighed 300 Gm. and showed severe mitral stenosis. All arteries, including those of the brain, were delicate and patent. Scattered areas of softening were present in the inferior portion of the right temporal, parietal and occipital cortex and the subjacent white matter. Several convolutions of the lateral portion of the right frontal lobe and the base of the left frontal lobe were firm, brown or yellow, and narrowed. The right lenticular nucleus showed anemic softening, and the left was cystic. The brain stem was intact. The basal arteries showed concentric and circumscribed webby fibrocellular proliferations of the intima, swollen elastic membranes and patent lumens. A small vein in the cortical softening contained a nodular dense fibrous and cellular intimal projection; another vein was thickly incased by dense collagen, and a third was filled by a laminated thrombus.

CASE 2.—In a white woman of 49 years acute polyarthritis developed two weeks after an attack of severe tonsillitis. The heart then gave evidence of fibrillation and decompensation. The patient became delirious. Her blood pressure was 175 systolic and 120 diastolic. The duration of the previous rheumatic heart disease was unknown.

The heart weighed 670 Gm. The mitral valve was dilated, and the cusps were thickened and bore verrucae. The aortic valve was moderately fibrosed. A thrombus was present in the right atrial appendage. There was active rheumatic myocarditis. The systemic arteries showed little sclerosis; the vessels of the brain were delicate. A typical area of "granular atrophy" of the second and third con-

10. Van der Horst, L.: *Digest of Neurol. & Psychiat.* **15**:399, 1947.

11. Rojas C., A., and Vila S., G.: *Rev. chilena de neuropsiquiat.* **1**:133, 1947.

volumens of the right frontal lobe measured 3 by 2 cm. There was a small old infarct in the right posteentral region. The small leptomeningeal arteries and venules of the affected area were thin walled, but the lumens were filled with acellular fibrillar connective tissue that had proliferated from the intima; loose adventitial connective tissue was abundant. Some superficial cortical capillaries were distended by homogeneous acidophilic material.

CASE 3.—A hypertensive white woman of 70 years died three weeks after suffering right hemiplegia. Rheumatic heart disease had not been diagnosed.

The heart was somewhat enlarged and slightly dilated. Moderate mitral stenosis with focal calcification and slight aortic valvular fibrosis were present. Other findings included mild coronary and aortic sclerosis and severe arterial and arteriolar nephrosclerosis. The vessels of the brain were practically free from sclerosis. A well defined, almost cystic infarct occupied the left parietal white matter, a part of the frontal lobe and the internal capsule. The middle cerebral artery contained a spongy fibrinous thrombus which enmeshed a few foam cells, was covered by endothelial cells and was attached by narrow strands to the intima in many places. The intima consisted of a narrow strip of fibrous tissue with few nuclei. The thickened homogeneous internal elastic membrane was superficially jagged. The media was diffusely fibrosed. The adventitia was slightly to moderately thickened and poorly nucleated.

CASE 4.—A white 39 year old woman had eclampsia and left hemiplegia nine years prior to consultation. She recovered from the paralysis, but it recurred two years ago. After that she had many epileptiform seizures. Death occurred five days after a third cerebrovascular accident. Presumably she had rheumatic fever without involvement of joints in childhood. The blood pressure was elevated.

The heart weighed 320 Gm. and showed chronic verrucose endocarditis and severe mitral stenosis. There was also myocardial and renal rheumatic vasculitis. The brain showed an old cystic infarct of the right frontoparietal area and a recent infarct of the left parietal lobe. The cerebral vessels were delicate, but the left middle cerebral artery was occluded by a soft thrombus. Histologically, the thrombus was a meshwork of fibrin covered by endothelium-like cells and attached to points of focal intimal thickening. Cellular and fibrous papillary nodules projected into the lumens of the larger vessels from the intima; the internal elastic membrane was swollen, irregular and split. The other arteries showed loose webby connective tissue filling the lumens, irregularity of the elastic membranes and hyaline thickening of the adventitia. One large artery was filled by a cellular-fibrous, extensively recanalized thrombus. The media was intact. Small arteries and veins in the area of the recent infarct showed intact intima but great compression of the media by hyalinized adventitia. Some of these vessels were also filled by loose connective tissue. Intracortical precapillaries with swollen endothelial and adventitial cells contained homogeneous fibrin-like masses in their lumens.

CASE 5.—A Mexican man of 60 years entered the psychopathic hospital because of recent persecutory delusions, assaultiveness and irritability. He had undergone three cerebrovascular accidents in the last three years, resulting in right hemiplegia. He died in cardiac failure, but rheumatic heart disease was not suspected.

The heart weighed 600 Gm. The aortic leaflets were fused, thickened and nodularly calcified. The free margin of the mitral valve was slightly thickened. The coronary arteries and the aorta were moderately sclerotic. The vessels of the brain were thin walled with smooth intima. A thrombus occluded the left middle cerebral artery. A softening of the left parietal lobe extended into the frontal and temporal lobes. This lesion appeared to be superimposed on an older cystic infarct.

The left half of the pons and the medulla was atrophied. The basal vessels showed diffuse concentric fibrous and cellular thickening of the intima with occasional focal mucinous areas and thick collagen bundles. The internal elastic membrane was thickened, glassy and homogeneous. A papillary, cushion-like intimal thickening projected into the lumen of the left middle cerebral artery, which also contained a recent porous fibrin thrombus. The media of this vessel, in part, was diffusely fibrosed and poorly nucleated. The adventitia of the veins was greatly thickened, and the endothelium was intact.

CASE 6.—A white man of 46 years had suffered from rheumatic heart disease for thirty-two years. Death followed the amputation of one leg because of embolic gangrene. There had been no symptoms referable to the brain.

The heart weighed 495 Gm. Both the aortic and the mitral valve were severely stenosed, and mural thrombi were present in both atriums. The cerebral vessels were grossly intact; a small scar was present in the cerebellar cortex. The large and small arteries of the thickened, edematous leptomeninges of the parietal lobe had numerous nodules protruding into their lumens. The lesions were composed of compact fibroblasts and some macrophages, were covered by endothelium and contained occasional capillary channels. Stains for elastic tissue revealed the formation of elastic laminae within the nodules. With the Van Gieson stain the nodules were yellow, and none contained collagen. The lumen of an occasional arteriole was completely blocked by hyaline thrombotic material, and the walls of some of the arterioles showed a mild increase in both collagenic and elastic fibers. The markedly thickened adventitia of a few vessels, together with the abundant perivascular connective tissue, contained proliferative fibroblasts and a moderate number of infiltrating lymphocytes with a few polymorphonuclear leukocytes. Several small arteries contained spherical acidophilic acellular nodules; these rested on thin intact intima, were free of elastic fibers and were covered by endothelium. Focal areas of the subjacent cortex were rarefied, depleted of neurons and contained gitter cells. In the cerebellar scar the folia were shrunken; most Purkinje and granular cells were absent; the Bergmann cell layer was spared.

CASE 7.—The heart of a white man of 60 years became progressively decompensated for one month, and he expectorated blood-tinged sputum during his last four days. Death was due to generalized miliary tuberculosis and rheumatic heart disease. The heart weighed 510 Gm.; the mitral valve was severely stenotic, and the cusps bore bland vegetations. There was mural thrombosis of the left atrium. The systemic arteries showed minimal sclerosis; the vessels of the brain showed none at all. Several small leptomeningeal arteries and a vein, each with intact walls, contained partially obstructive homogeneous acidophilic masses covered by flat endothelium-like cells. The wall of a large vein possessed greatly thickened fibrous adventitia and intact endothelium. The cortical cells showed ischemic changes. Tuberculous lesions of the central nervous system were absent.

COMMENT

The vascular lesions observed in our cases may be summarized as follows: The vessels chiefly involved were the small, medium-sized and larger basal arteries and also the veins of the leptomeninges. In 3 cases the lumen of a large vessel contained, but was not completely obstructed by, a spongy polypoid thrombotic mass made up predominately of fibrin and covered by a layer of flat endothelial cells. Thrombi of this type sometimes showed admixture of erythrocytes and small

collections of foam cells. Unlike the thrombi typically seen in other conditions, these resembled the cardiac valvular vegetations that are present in rheumatic endocarditis. At the points of their attachment to the intima, endothelial lesions were minimal or absent in some cases, but in others there were circumscribed cellular-fibrous intimal thickenings. In addition to the massive thrombi, small solid sessile thrombi were observed, predominately in smaller vessels. In later stages these small thrombi were converted into richly cellular nodules with occasional elastic fibers and capillaries, but without collagen. Apart from thrombosis, there was apparently independent intimal proliferation of fibroblasts and fibers arranged in a loose webby fashion; mucinous foci and occasional collagenic bundles showing fibrinoid degeneration were present. This endarteritic process usually left the lumen patent but in a single instance produced complete obstruction with recanalization. It is open to question whether the rarefied appearance of the intimal and luminal connective tissue might be the final outcome of resorption of hyaline or lipid material, similar to the process described by Scholz and Nieto¹² in hyalinosis of cerebral arterioles. The internal elastic membrane was constantly altered, showing homogeneous thickening, splitting and disruption, and superficial jagged indentations. The media showed fibrosis with close intermingling of the collagen and smooth muscle fibers. The adventitia was thickened frequently, owing to overgrowth of collagenic tissue.

An important question is whether the thrombi mentioned previously have always formed *in situ* or whether some of them are embolic. In all of our cases there were old valvular lesions: in some, recurrent mitral vegetations, and in 2, mural thrombi in the left cardiac chambers. There was embolism of the lower extremity in 1 instance. At first sight this might suggest an embolic genesis of the cerebrovascular occlusions. It is doubtful, however, whether firmly adherent valvular vegetations of chronic rheumatic heart disease ever become embolized. Probably emboli from mural thrombi would block larger vessels, causing complete occlusion. It would appear, therefore, that masses of fibrin seen in smaller vessels, as in our cases, are thrombotic rather than embolic. This conclusion is supported by their attachment to vessel walls only in circumscribed areas and by their incomplete obstruction of the lumen. Frequently, one gets the impression that the larger thrombi mushroom from a comparatively minute point or points of intimal attachment. In their histologic structure also they differ from emboli, because they consist predominantly of a fibrin meshwork with a paucity of cellular elements rather than being solid laminated thrombi. The genesis of the

12. Scholz, W., and Nieto, D.: *Ztschr. f. d. ges. Neurol. u. Psychiat.* **162**:675, 1938.

process, in accordance with the views of Roessle,¹³ may have been as follows: The subendothelial connective tissue underwent fibrinoid swelling, the endothelial layer was loosened and broken away from its



Fig. 1.—Loosely attached polypoid thrombus in a large artery. $\times 55$.

Fig. 2.—Recent mural fibrinous thrombus and an intimal hillock, possibly an organized thrombus. $\times 60$.

base, plasma and cellular elements from the lumen were pressed in, and thus a subendothelial cushion was formed. It is possible that the gaps

13. Roessle, R.: *Virchows Arch. f. path. Anat.* **288**:780, 1933.

between the endothelial layer and the remainder of the vessel wall, frequently seen in our cases, were not, or were not necessarily, artefacts but were rather an intravital process identical with Roessle's "decollation."

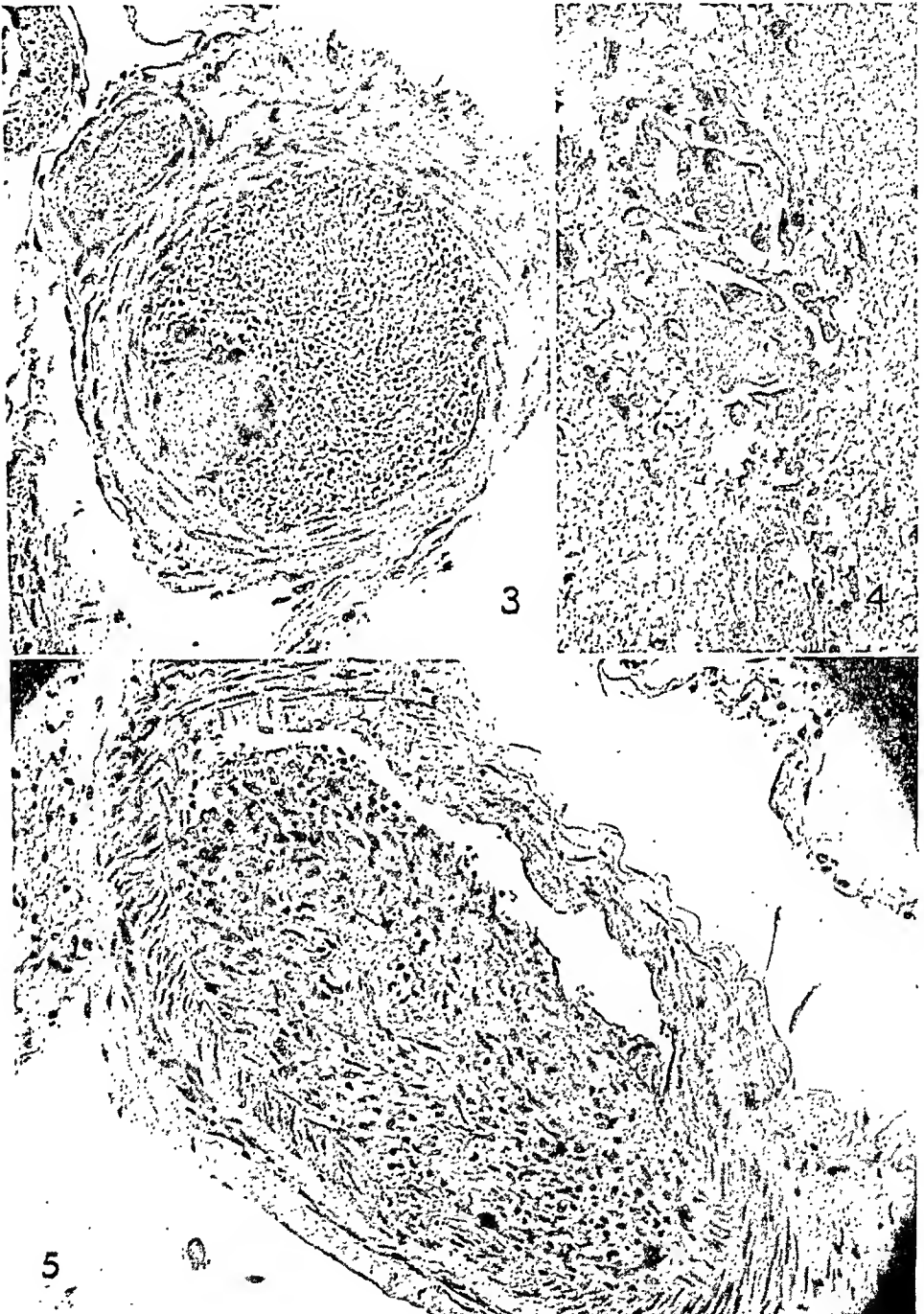


Fig. 3.—Discrete mural thrombus in a small leptomeningeal artery. $\times 200$.

Fig. 4.—Thrombosis of intracortical precapillaries. $\times 325$.

Fig. 5.—Subtotal obliteration of the lumen by an organized thrombus or possibly an intimal proliferation. $\times 150$.

tion and desmolysis." The adventitia often showed fibrous thickening, occasionally of a high degree and associated with compression of the media.

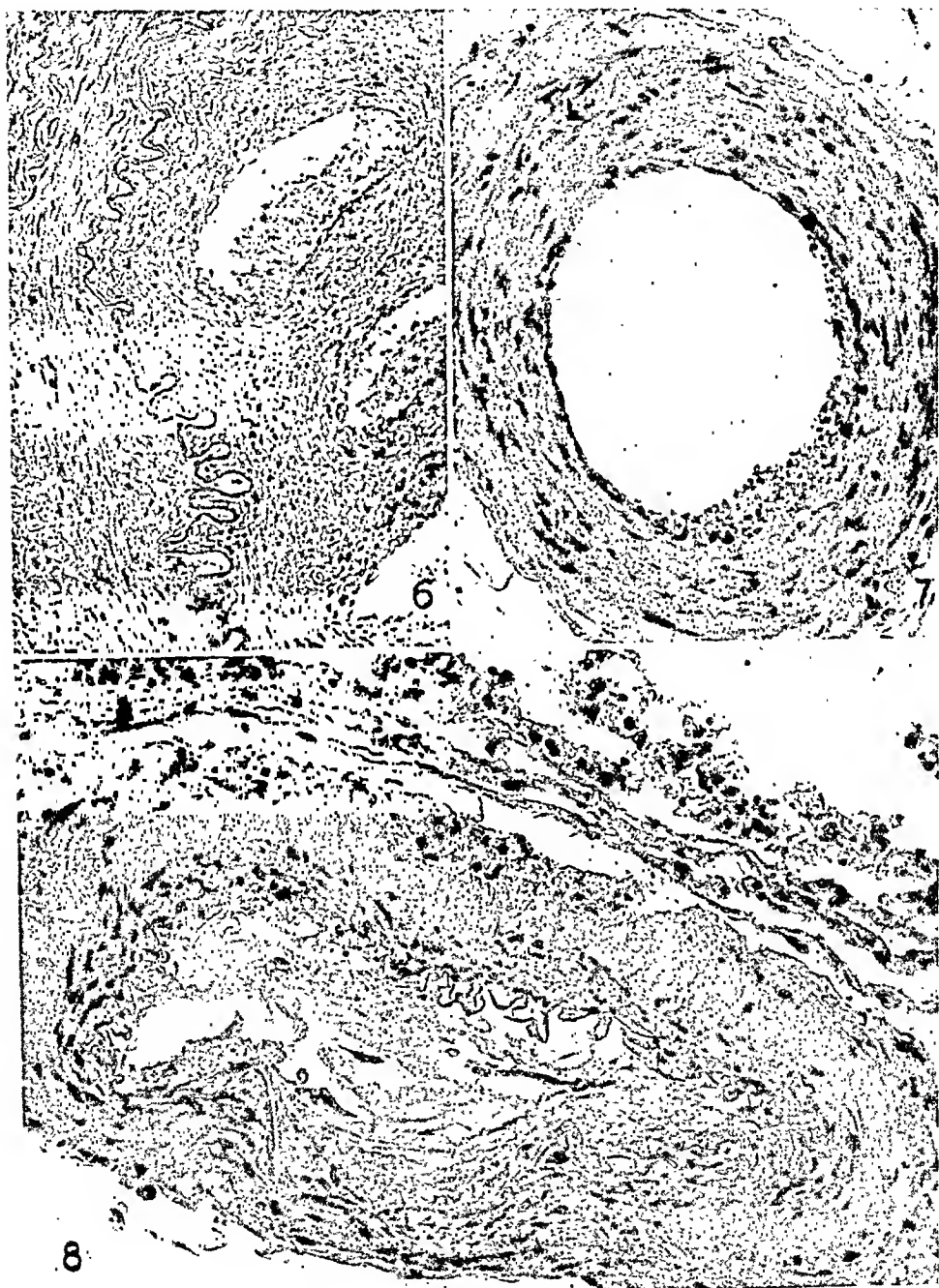


Fig. 6.—Recanalization of connective tissue filling the lumen of a large artery; fibrosis of outer coats. $\times 125$.

Fig. 7.—Endarteritis with concentric intimal proliferation. Note the internal elastic membrane and the thin media. $\times 180$.

Fig. 8.—Small artery with loose connective tissue filling the lumen and with adventitial fibrosis. $\times 175$.

The lesions of the nervous parenchyma secondary to the vascular alterations were not essentially different from those produced by other forms of circulatory disturbance and will not be discussed. All lesions from minute cerebral infarcts and scars to large softenings with formation of cysts were observed, and the occurrence of so-called granular atrophy of the cortex is probably not infrequent.

The differentiation of vascular lesions associated with the late stage of rheumatic heart disease from other vascular lesions found in the cranial cavity requires discussion. The rheumatic alterations histologically are not like those of arteriosclerosis. Arteriosclerosis was grossly absent in every case. The peculiarities of the thrombi, the lack of atheromatous plaques and the presence of greatly thickened adventitia are other distinguishing features.

"Obliterative cerebral arteriosclerosis" as described by Scheinker¹⁴ shows "tremendous expansion of the intima resulting in narrowing or complete obliteration of the vessel lumen." The absence of endothelial proliferation and of fibrin plugs distinguishes this condition from rheumatic lesions, according to Scheinker.

Vascular lesions of thromboangiitis obliterans (Buerger's disease), which in rare instances may involve the central nervous system, may be similar to some of the rheumatic manifestations; however, in such cases the absence of rheumatic heart disease and the presence of Buerger's disease elsewhere would be distinguishing.

Cerebrovascular lesions found in hypertension, even without arteriosclerosis, consist of loosening, edema, fatty metamorphosis, partial necrosis, fibrosis and hyaline degeneration of the arteriolar walls of the brain and the meninges.

In syphilis the intimal proliferation commonly is accompanied by adventitial inflammation or even lymphocytic infiltration of all layers; thrombi are absent.

In periarteritis nodosa the essential findings are heavy segmental inflammation, intimal and medial fibrinoid degeneration and necrosis, and perivascular fibrosis.

While, therefore, the vascular changes in the meninges and the brain accompanying rheumatic heart disease appear to be different from those in other conditions, occasional diagnostic difficulties will be encountered if the microscopic picture alone is considered. The "specificity" of those lesions is debatable. Because the human body has at its disposal a limited number of tissue reactions that may be elicited by a host of causative agents, it is not surprising that almost identical histologic changes occur in etiologically different conditions. It is often impossible to give a succinct and incontestable statement as to the nature of a

14. Scheinker, I. M.: *Am. J. Path.* 22:565, 1946.

lesion solely on the grounds of the histologic structure, without knowledge of the over-all findings in the respective case. It follows that some of the vascular lesions described may occur in other conditions, too, but they dominate the picture in such a "characteristic" if not "specific" way in cases of rheumatic heart disease that they may be designated as rheumatic lesions. Therefore, the term "rheumatic brain disease" as employed by Bruetsch appears justified.

We have expressed our belief that while most of the vascular lesions are due to thrombosis and organization, undoubtedly there are others that are characterized by independent endarteritic proliferation. In early stages the differentiation is easy. In late stages it is sometimes impossible to decide whether the plug filling the lumen is the result of organization of a thrombus or the result of endarteritis. This difficulty was also encountered by Lindenberg and Spatz¹⁵ in their attempt to explain the genesis of the arterial lesions of Buerger's disease involving the brain.

The cerebrovascular condition was a decisive factor in causing death in 2 patients, both of whom had hypertension. The presence of hypertension failed to manifest itself in characteristic cerebrovascular lesions, and consequently hypertension was not considered the important pathogenic factor. In other cases, the brain lesions were incidental observations at autopsy, or were observed only microscopically. In the majority of cases, death was due to the cardiac sequelae of rheumatic heart disease.

Most of the vascular lesions described, particularly the thrombi, appeared to be fairly recent. Their development in late stages of exceedingly chronic rheumatic heart disease, comparable to the events in syphilis, can be understood only when the cause of rheumatic fever is clarified. It is possible that deterioration of circulation in the final stages of cardiac failure favors the development of the lesions. The medial fibrosis is probably the outcome of an older process; it may be related to scars in the media of other vessels as described by Von Glahn and Pappenheimer,¹⁶ Klinge and Vaubel,¹⁷ and others. The late lesions are different from those described in cases of more acute rheumatic disease, in which cortical capillary endangiitis (Winkleman and Eckel⁴) and hyalinization of intracerebral vessels, adventitial fibrosis and perivascular fibrocytic nodules in meningeal vessels (Neubuerger¹⁸) may occur; the only finding common to acute and chronic stages is adventitial fibrosis. All features observed are consistent with the various forms of "acute inflammations of arteries" that were described by

15. Lindenberg, R., and Spatz, H.: *Virchows Arch. f. path. Anat.* **305**:531, 1940.

16. Von Glahn, W. C., and Pappenheimer, A. M.: *Am. J. Path.* **2**:235, 1926.

17. Klinge, F., and Vaubel, E.: *Virchows Arch. f. path. Anat.* **281**:701, 1931.

18. Neubuerger, K. T.: *Dis. Nerv. System* **8**:259, 1947.

Karsner¹⁹ as occurring in the course of rheumatic fever, as well as with lesions of myocardial vessels seen in active and inactive forms of the disease by Gross, Kugel and Epstein.²⁰ However, they are not identical with the typical inflammatory picture of generalized rheumatic arteritis (Von Glahn and Pappenheimer¹⁶; Klinge and Vaubel¹⁷).

With regard to the clinical significance of the vascular lesions we should like to make the following statement: Focal neurologic symptoms will occur when the vascular changes are sufficiently developed to cause circulatory disturbances in important areas. Infarctions, softening cysts, and granular atrophy may or may not manifest themselves clinically; this will depend on the number and location of the destroyed areas in the parenchyma in a given case. We believe that the lesions under discussion are not the anatomic basis for psychoses, particularly schizophrenia. Lesions absolutely identical with those described by Bruetsch may be found in both psychotic and nonpsychotic patients. Whether a patient with such lesions becomes schizophrenic or epileptic depends mainly on his own psychic makeup and other factors yet unknown; focal occlusions of smaller or larger intracranial vessels, with ensuing focal parenchymal lesions, may or may not be a contributory factor. As recently emphasized by Moersch²¹ in the discussion of Bruetsch's last paper, "it is not known how numerous or how extensive lesions in the brain must be for the production of mental symptoms."

SUMMARY

Lesions of intracranial vessels occur with unexpected frequency in late stages of rheumatic heart disease. The larger and smaller arteries and veins, mainly in the leptomeninges, show a great variety of pathologic features. The principal lesions are: various forms of thrombosis, endarteritic proliferation, alteration of the elastic membranes, and fibrosis of media and adventitia. The histologic picture, as a rule, is sufficiently characteristic to permit differentiation from other vascular diseases that occur in the same location. Parenchymal changes in the brain secondary to the vascular processes are identical with those produced by other vascular diseases. Focal neurologic signs may occur, but there is little evidence to show that the lesions described form the anatomic basis for psychotic syndromes.

19. Karsner, H. T.: *Acute Inflammations of Arteries*, Springfield, Ill., Charles C Thomas, Publisher, 1947.

20. Gross, L.; Kugel, M. A., and Epstein, E. Z.: *Am. J. Path.* **11**:253, 1935.

21. Moersch, F. P.: *J. A. M. A.* **134**:453, 1947.

MYOCARDIAL CHANGES IN POLIOMYELITIS

VERA B. DOLGOPOL, M.D.

AND

MARY D. CRAGAN, M.D.

NEW YORK

THE OCCURRENCE of myocarditis in poliomyelitis was recognized in 1941, although clinical evidence of myocarditis and of cardiac irregularity had been reported prior to that date by two observers. Hertz, Johnson and Depree,¹ in 1913, reported a case of myocarditis of several months' duration, with recovery. Neal,² in 1932, mentioned briefly several fatal cases with rapid pulse, and with cyanosis persisting despite the use of the respirator, and 1 case with intervals of great cardiac irregularity; in these cases death was attributed to the involvement of cerebral vital centers.

The first report on microscopic evidence of myocarditis in poliomyelitis was that of Larson,³ in 1941, who encountered myocarditis in 2 of 12 necropsies of that disease. These cases were described in detail two years later by Dublin and Larson.⁴ In 1942, before the appearance of the latter paper, Saphir and Wile⁵ reported that myocarditis had been observed in 6 of 7 necropsies of infantile paralysis. In the following year three papers appeared: the detailed report of Dublin and Larson⁴; a paper by Peale and Lucchesi,⁶ who reported acute myocarditis observed in 5 of 9 cases of poliomyelitis, and Hassin's⁷ report of a case. In a more recent article Saphir⁸ encountered myocarditis in 10 of 17 necropsies of poliomyelitis.

From the Pathologic Laboratories, Willard Parker Hospital.

1. Hertz, A. F.; Johnson, W., and Depree, H. T.: *Guy's Hosp. Rep.* **67**:105, 1913.

2. Neal, J. B., in *Poliomyelitis: International Committee for the Study of Infantile Paralysis*, Baltimore, Williams & Wilkins Company, 1932, pp. 175 and 176.

3. Larson, C. P.: *Northwest Med.* **40**:448, 1941.

4. Dublin, W. B., and Larson, C. P.: *Am. J. Clin. Path.* **13**:15, 1943.

5. Saphir, O., and Wile, S. A.: *Am. J. M. Sc.* **203**:781, 1942.

6. Peale, A. R., and Lucchesi, P. F.: *Am. J. Dis. Child.* **65**:733, 1943.

7. Hassin, G.: *J. Neuropath. & Exper. Neurol.* **2**:293, 1943.

8. Saphir, O.: *Am. J. Path.* **21**:99, 1945.

In an earlier description of myocarditis in a case of infantile paralysis, in 1937, Clark,⁹ attributed the cardiac lesions to serum disease and serum carditis following administration of horse serum.

Electrocardiographic studies of patients with poliomyelitis also showed evidence of cardiac damage in that disease. Battro, Cibils Aguirre and Mendy,¹⁰ in Argentina, found transient myocarditis in 4 of 20 patients in the acute stage of poliomyelitis, and none in 18 in the chronic stage. Morrow¹¹ has observed abnormal electrocardiographic tracings of some convalescent patients in the Poliomyelitis Division of the Knickerbocker Hospital in New York.

All pathologic reports showed a high incidence of acute myocarditis in fatal cases of infantile paralysis. However, each report was based on a small number of cases which did not permit a fair estimation of the actual incidence of this complication.

The present report was undertaken for the purpose of establishing the incidence of acute myocarditis in poliomyelitis in the large pathologic material of the Willard Parker Hospital. The material available in the files, covering the years from 1929 to 1947 inclusive, exceeded by far the combined number of cases reported to date in the literature. Sections of the myocardium were available from the majority of the old, and from all the more recent, complete necropsies, and acute myocarditis had been recorded as the cause of death in several cases.

MATERIAL

The present report is based on microscopic study of the material from 92 patients with poliomyelitis. All but 1 were in the acute or the convalescent stage of the disease; 1 patient, ill for ten months, was considered as having a chronic involvement.

Sections from the myocardium were searched for lesions of acute myocarditis; the lungs were examined for pulmonary changes, and the pathologic and clinical observations were correlated. Only cases with acute focal myocardial lesions were considered as positive for the complication in question. Cases of rheumatic heart disease were recorded as negative.

Acute focal myocarditis was found in 16 of 92 cases of poliomyelitis examined. The duration of poliomyelitis in the cases with myocardial lesions was from two to ten days, and in the majority of them (12 cases) did not exceed five days. The age of the patients ranged from 13 months to 37 years. Death from cardiac failure was recorded in 4 cases. In 2 other cases the clinical picture was suggestive of cardiac damage. Two patients had received intravenous injections of antipoliomyelitis horse serum one and three days before death, respectively. In all cases bulbar and spinal involvement of varying severity was observed microscopically, and in several, also involvement of the midbrain and the cortex.

Brief abstracts from 8 clinical histories are presented.

9. Clark, E.: *J. A. M. A.* **110**:1098, 1938.

10. Battro, A.; Cibils Aguirre, R., and Mendy, J. C.: *An. d. Inst. de invest. fisiol.* **5**:7, 1943.

11. Morrow, D. J.: Personal communication to the authors.

CASES OF POLIOMYELITIS WITH CLINICAL EVIDENCE OF CARDIAC FAILURE

CASE 1.—A white boy 13 months old was admitted on the second day of an illness characterized by malaise, a temperature of 101.6 F. and paralysis of one leg only. The spinal fluid contained 230 cells. On the fourth day of illness he became cyanotic and apparently died of heart failure. Necropsy note: no pneumonia.

CASE 2.—A white girl $3\frac{1}{2}$ years old was admitted on the third day of an illness characterized by headache, stiff neck, fever, vomiting and weakness of the muscles of the left leg. The spinal fluid contained 560 cells. On the fifth day of illness she died apparently of cardiac failure. Necropsy note: no pneumonia.

CASE 3.—A white boy 14 years old was admitted on the third day of an illness characterized by fever (temperature 103.2 F.) headache, pain in the neck, vomiting, inability to swallow, nasal voice and palsy of the right side of the face. The extremities were intact. The spinal fluid contained 310 cells. On the fourth day of illness the temperature was 105.6 F., the pulse was rapid, the heart sounds were of poor quality and a systolic murmur was heard over the precordium; the patient died a few hours later. Necropsy note: no pneumonia.

CASE 4.—A Negro boy 6 years old was admitted on the third day of illness. He had a headache, pain in the back of his neck, paralysis of the left side of the face and a temperature of 104.4 F. The spinal fluid contained 330 cells. On the fourth day he was unable to swallow. On the fifth day the pulse became irregular. This continued for three days. On the eighth day of illness the patient died of cardiac failure, with the heart stopping before the respirations ceased. Necropsy note: hypostatic pneumonia.

CASE 5.—A white boy $8\frac{1}{2}$ years old was admitted with a history of headache and fever for the past six days and of nasal voice since the preceding day. On admission there was difficulty in swallowing and a temperature of 104 F. The spinal fluid contained 85 cells. The pulse rate changed intermittently from 110 to 140. On the seventh day of illness the patient was semicomatose. He died on the tenth day of illness. Necropsy note: pneumonia in a few alveoli in one section only.

CASE 6.—A white girl 11 years old was admitted on the fourth day of an illness which was characterized by vomiting followed by inability to sit up or turn her head. On admission she had nasal speech, weakness of the left side of the face and palate and bilateral weakness of the sternocleidomastoid muscle. Her mouth was full of blood-tinged mucus and froth. Her temperature was 101 F.; the pulse rate was 110 to 120; the blood pressure, 146 systolic and 100 diastolic. On the fifth day she suddenly ceased to breathe. Necropsy note: hemorrhagic infarct in one lung; mucus in bronchi, but no bronchitis or pneumonia.

CASES IN WHICH PATIENTS WERE TREATED WITH IMMUNE HORSE SERUM

CASE 7.—A white boy 14 years old was admitted on the second day of an illness characterized by pain in the back and the neck, vomiting, and weakness of the left arm. The temperature was 103 F. The spinal fluid contained 310 cells. He received on admission 10 cc. of immune horse serum intravenously. Shortly thereafter, a headache and chill developed and the temperature rose to 104.4 F. On the following day the patient had involvement of intercostal muscles and was placed in the respirator, but he became cyanotic and unconscious and died a few hours later, on the third day of illness and one day after the administration of the horse serum. Necropsy note: no pneumonia.

CASE 8.—A white girl 8 years old was admitted on the second day of illness with pain in the back, headache, rigidity of the neck and a temperature of 102.6 F. The spinal fluid contained 130 cells. The diagnosis was preparalytic poliomyelitis, and 10 cc. of immune horse serum was given intravenously. Two days later, there was weakness of the right leg, and another 10 cc. of immune horse serum was given intravenously. One and one-half hours later the patient had a flushed face, was dyspneic and showed some involvement of intercostal muscles. On the next day, the fifth day of the disease, and three days after the first dose of horse serum, she died, apparently from respiratory paralysis. Necropsy note: no pneumonia.

Of the remaining 8 patients, 1 died of pneumonia and the rest with manifestations of either bulbar or respiratory paralysis.

PATHOLOGIC CHANGES IN THE MYOCARDIUM

Focal Myocarditis.—Three types of focal myocardial lesion were observed.

The first type of lesion was the least conspicuous. At low magnification it appeared as areas containing a greater number of nuclei than the rest of the myocardium (fig. 1 *A*). At high magnification one could see in those areas isolated thin and cloudy myocardial fibers, tapering off toward the widened, edematous stroma, and long, wavy nuclei clinging to the fibers (fig. 1 *B*). No cytoplasm could be distinguished around those nuclei, which apparently did not belong to cells of hematogenous origin. In some cases the cells seemed to originate from the endothelium of the capillaries, while in others they appeared to derive from the interstitial stroma of the myocardium. This lesion was seen best in longitudinal sections of the muscle fibers. This type of lesion was noted in 5 cases.

The second type of lesion consisted of cellular collections between intact or only slightly degenerated myocardial fibers. The infiltrating cells usually were mononuclear, with only occasional polymorphonuclear ones. In some foci the mononuclear cells were all lymphocytes (fig. 1 *C*). In others the mononuclear cells had elongated or oval nuclei and little cytoplasm and seemed to be of histiocytic origin (fig. 1 *D*). The character of infiltrates in a given block of tissue was fairly constant, but in lesions located in distant parts of the same heart the character of cells was often variable. In 1 case, for instance, the infiltrates in the wall of the left ventricle consisted of elongated histiocytes, while in the wall of the left auricle all infiltrating cells were lymphocytes. This type of lesion was seen in 8 cases.

The third type of lesion consisted of cellular collections in the interstitial tissue surrounding the blood vessels and was encountered as an independent lesion or in combination with the preceding types. The cells usually were histiocytic mononuclears with oval nuclei (fig. 2).

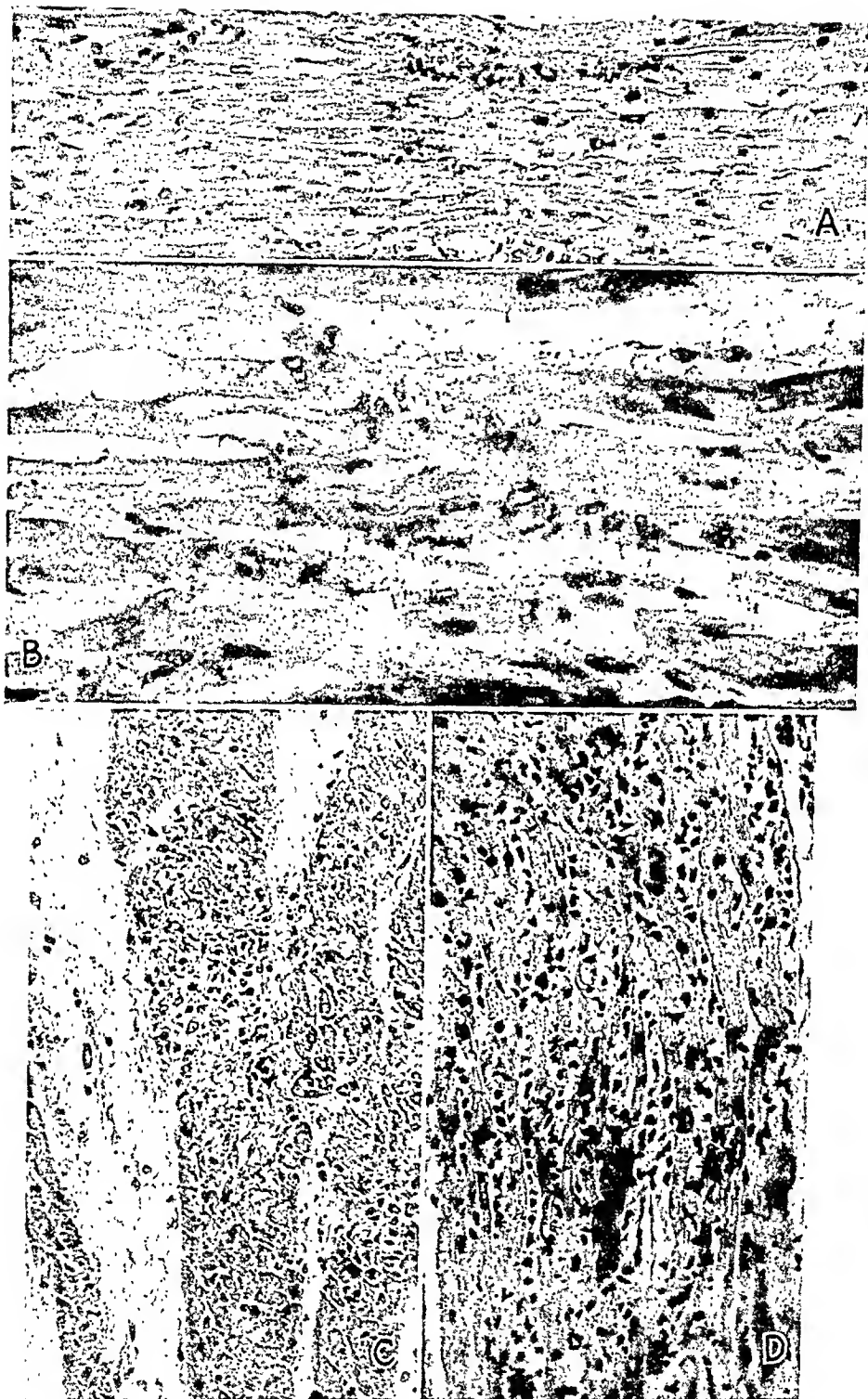


Fig. 1.—*A*, mononuclear cells clinging to individual myocardial fibers in case 5 ($\times 220$).

B, similar lesion from another case under higher magnification ($\times 440$). Note the elongated and wavy nuclei at the borders of degenerated, thin, tapering myocardial fibers.

C, lymphocytic collections in myocardial bundles; interstitial edema in case 1 ($\times 220$).

D, collections of mononuclear cells with oval nuclei, and some lymphocytes, between slightly damaged myocardial fibers in case 4 ($\times 220$).

but in 2 cases polymorphonuclears and lymphocytes predominated in the perivascular tissue and infiltrated between the adjacent muscle fibers (fig. 3 *A*).

In several cases small collections of lymphocytes and polymorphonuclears were observed beneath the endocardium, sometimes spreading between the underlying myocardial fibers. Subepicardial cellular collections were frequent, especially in the cases with epicardial petechiae. Petechiae were also frequent within the myocardium and beneath the endocardium.

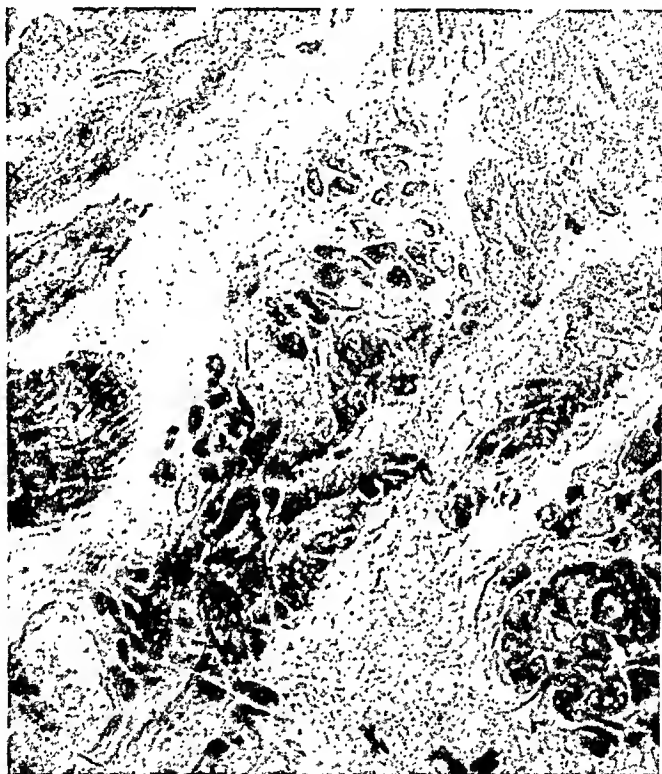


Fig. 2.—Interstitial perivascular collections of histiocytic cells in case 2 ($\times 440$).

The individual inflammatory foci were small, and several foci were found in one section. However, foci of myocarditis were only rarely encountered in more than two blocks of tissue from the same heart, a fact indicating that the process was not diffuse. The lesions were most frequently located in the posterior wall of the left ventricle and in the posterior papillary muscle, but occasional small foci were encountered in the wall of the left auricle and in the interventricular septum.

There was no relation between the number and severity of focal myocardial lesions and the duration of poliomyelitis or the presence of inflammatory changes in the lungs. The majority of patients with myocarditis died within the first five days of illness. The length of illness

and the pulmonary pathologic conditions in the 16 cases of myocarditis are listed in tables 1 and 2.

Pneumonia was observed in 4 cases, but in 2 it presented the characteristics of hypostatic pneumonia and could perhaps be regarded as a sequel rather than the cause of myocarditis. In 4 cases there were only minimal inflammatory changes in the lungs, and in the case of the hemorrhagic infarct, again, the lesion was to all evidence the result of myocarditis. No inflammatory changes were found in the lungs of 8 patients with myocarditis.

Interstitial Edema.—Interstitial edema of the myocardium was present in all but 6 of the 92 hearts examined. In many cases it not

TABLE 1.—*Length of Illness*

Days	Cases
2.....	1
3.....	2
4.....	4
5.....	5
6.....	2
8.....	1
10.....	1
Total.....	16

TABLE 2.—*Pathologic Changes in the Lungs*

	Cases
Lobular pneumonia.....	2
Hypostatic pneumonia.....	2
Peribronchitis.....	2
Pneumonia in a few alveoli in one section only.....	1
Hemorrhagic infarct with some polymorphonuclear invasion.....	1
No inflammatory changes in the lungs.....	8
Total.....	16

only involved the perivascular connective tissue but extended within the muscle bundles, between individual myocardial fibers.

Degenerative Changes in the Myocardium.—Isolated thin and cloudy myocardial fibers, with mononuclear cells clinging to them, were present in the first type of focal myocarditis presented here, constituting the main feature of that lesion. However, in the majority of cases of myocarditis the heart muscle was remarkably well preserved. Zenker's type of degeneration was encountered occasionally in isolated fibers in several specimens, usually in cases without focal myocarditis. A peculiar widespread degenerative change was observed in some cases with or without myocarditis. The degenerated fibers, when casually examined, appeared to be hydropic because of empty spaces within the sarcolemma. On closer examination, however, it became evident that the lesion consisted of intrasarcolemmal fragmentation of the fibrillae.

The fibrillae, still preserving their cross striation, appeared to be cleaved across the intercalated disks and retracted on either side of the tear while the intact sarcolemmal sheath over the gap became partly collapsed after the recession of the contractile components of the myocardial fiber (fig. 3 *B*). This fragmentation differed significantly from intrasarcolemmal fragmentation of the myocardium observed in the early, degenerative phase of diphtheritic myocarditis, because in the latter disease the fragmented muscle shows Zenker's degeneration with swelling and fusion of the fibrillae and complete obliteration of their striation.

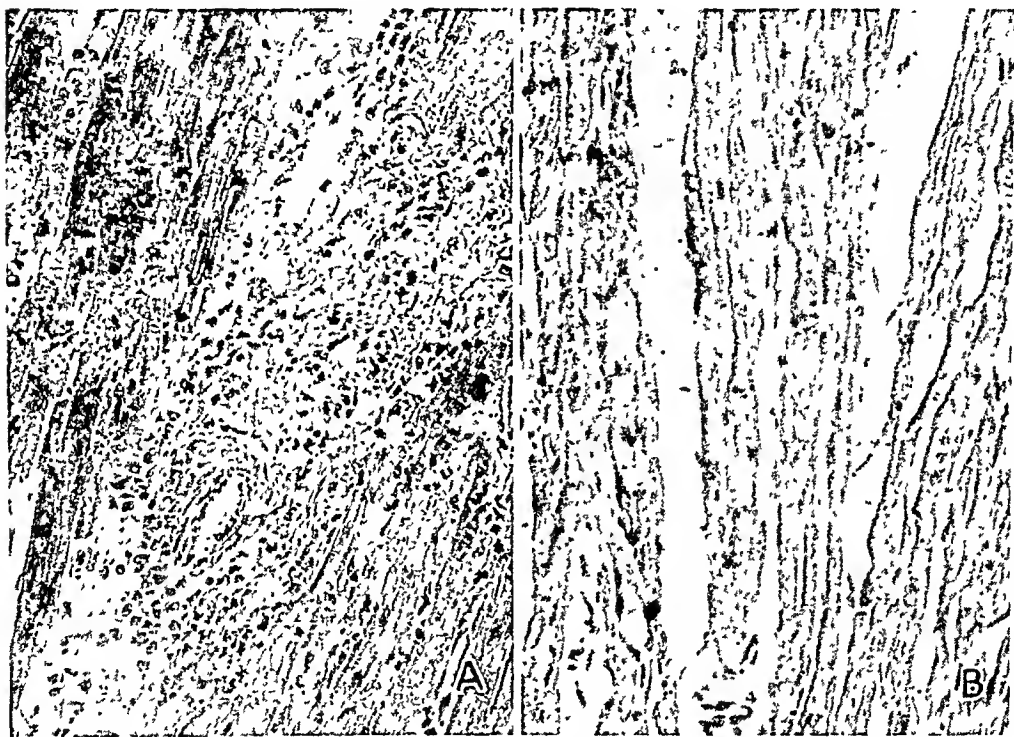


Fig. 3.—*A*, interstitial collections of polymorphonuclears, with some infiltration between intact myocardial fibers in case 7 ($\times 220$). The artery is intact.

B, intrasarcolemmal fragmentation of myocardial fibers in a case in which there was no myocarditis ($\times 220$). The striation of the myofibrillae is largely preserved.

COMMENT

Of all lesions mentioned in the foregoing section, myocarditis is undoubtedly the most important. Focal myocarditis has been seen in other virus diseases—for example, in mumps (Manca¹²), in infectious mononucleosis (Allen and Kellner¹³), which presumably is a virus disease, and in influenza A infection (Finland and associates¹⁴). Myo-

12. Manca, C.: *Arch. ital. di anat.* **3**:707, 1932.

13. Allen, F. H., and Kellner, A.: *Am. J. Path.* **23**:463, 1947.

14. Finland, M.; Parker, F., Jr.; Barnes, M. W., and Jolliffe, L. S.: *Am. J. M. Sc.* **209**:455, 1945.

carditis of a virus origin has been produced experimentally in animals (Schmidt ¹⁵).

The etiologic relation of myocardial lesions and the virus of poliomyelitis cannot, obviously, be established on the basis of purely morphologic study and would require experimental corroboration. However, the analysis of our cases points to the possibility of such a relation. The only bacterial inflammatory process that could be expected to occur within the short duration of the poliomyelitis in our cases complicated by myocarditis (two to ten days) would be pneumonia and bronchitis. Stone ¹⁶ observed focal myocardial infiltrates in 13.5 per cent of cases of bronchopneumonia. However, bronchopneumonia was present in only 2 of our 16 cases, and bronchitis with peribronchial and minimal interstitial infiltration in 2 cases; the hypostatic pneumonia in 2 other cases could very well have been secondary to the myocarditis. In 8 cases no inflammatory process was found in the lungs or the bronchi, and in 2 the inflammatory changes were limited to a small portion of one microscopic field and were negligible in extent. The etiologic factor in these 10 cases was, therefore, probably nonbacterial. Moreover, the occurrence of myocarditis on the second and third day of poliomyelitis in 3 of our cases and the death of cardiac failure on the fourth and fifth day of illness in 3 other cases indicate that myocarditis may be a very early complication developing before bacterial invasion of the respiratory organs becomes sufficiently severe to lead, in turn, to focal myocarditis.

The 2 cases in which focal myocarditis occurred in patients treated with immune horse serum require a special discussion. Interstitial myocardial infiltrates and vasculitis in patients with serum disease following the administration of horse serum had been described by Clark and Kaplan ¹⁷ and the possibility of a similar reaction in our cases had therefore to be considered. However, in our cases myocarditis developed too soon after the administration of the serum to be the result of an allergic reaction, as the patients died one and three days, respectively, after the treatment, before serum disease could develop. The morphologic aspect of the myocardial lesions was also different from that of the lesions observed by Clark and Kaplan. No vasculitis was seen in either case. The interstitial cellular infiltrates in one case consisted predominantly of polymorphonuclears, while in the other case collections of lymphocytes were present between intact myocardial fibers. In the instances of serum carditis the predominant cells of perivascular infiltrates were histiocytes and fibroblasts. Neither of our patients had

15. Schmidt, E. C. H.: *Am. J. Path.* **24**:97, 1948.

16. Stone, W. J.: *Am. J. M. Sc.* **163**:659, 1922

17. Clark, E., and Kaplan, B. I.: *Arch. Path.* **24**:458, 1937.

pneumonia. The cause of myocarditis in these cases was, therefore, probably not different from that in other cases.

From the point of view of morphology the focal myocardial lesion in poliomyelitis is not uniform. The lesions of the first type described here are similar to those observed by Finland and associates in influenza A virus infection. Lesions of the second and the third type, with some modification of cellular components and degree of intensity, not only are encountered in a variety of bacterial and rickettsial diseases but have been seen in mumps and infectious mononucleosis. The myocarditis in infantile paralysis is of less extent than that in scarlet fever, diphtheria or rickettsial diseases. The damage to the muscle fibers in the foci of myocarditis in poliomyelitis is, as a whole, relatively small, especially as compared with that in diphtheria, in which severe Zenker's degeneration precedes the inflammatory infiltration.

The incidence of myocarditis in our material, observed in 16 of 92 cases, or 17.4 per cent, is lower than that recorded by other observers. However, it must be taken into consideration that in our material, largely taken from the files, few sections were available from individual specimens. In slightly over one half of our material, 47 cases, only one section was on hand. Acute focal myocarditis was seen in 5 cases, or in 10.6 per cent of that group. In the remaining 45 cases, with two to eight blocks from each specimen, myocarditis was encountered in 11 cases, or in 26.6 per cent. As mentioned before, the myocarditis was not diffuse, and it is obvious that multiple blocks afforded a better opportunity of finding the widely scattered focal lesions. We feel, therefore, that the latter figure of approximately 27 per cent is probably closer to the actual incidence of focal myocarditis in fatal poliomyelitis than the incidence calculated from our total material. The myocarditis may in some cases be regarded as the immediate cause of death.

SUMMARY

Focal myocarditis was found in 16 of 92 cases of poliomyelitis. The incidence of myocarditis in 45 cases in which multiple sections from each heart were available was 26.6 per cent. Cardiac failure was the immediate cause of death in at least 4 cases.

Three histologic types of lesions are described.

In most of the cases the myocarditis was observed between the second and the fifth day of illness. Pneumonia and other evidence of pulmonary inflammation were absent in one half of the 16 cases.

Myocardial interstitial edema was present in most of the 92 cases.

Intrasarcolemmal fragmentation of myocardial fibers with preservation of striation in the retracted fibers and collapse of the sarcolemmal sheath was seen in many cases in which no myocarditis was found, and in a few cases of myocarditis.

COMPARISON OF THYMIC HYPERPLASIA IN MYASTHENIA GRAVIS AND EXOPHTHALMIC GOITER*

ALLEN L. BRYAN, M.D.

Fellow in Surgery, Mayo Foundation

JOHN R. McDONALD, M.D.

AND

O. THERON CLAGETT, M.D.

ROCHESTER, MINN.

THE functions of the thymus remain undetermined, and there has been much controversy concerning metabolic disturbances attributed to thymic dysfunction. There is suggestive evidence that the thymus plays an endocrine role. Hyperplasia of the thymus occurs in acromegaly, hypofunction of the adrenal glands (Addison's disease), castration, hyperthyroidism and myasthenia gravis. Simpson and co-workers¹ found that in the rat adrenalectomy caused thymic enlargement, while, conversely, administration of an extract containing the adrenocorticotrophic factor of the pituitary gland caused an involutionary change in the thymus and the lymph nodes. Marine, Manley and Baumann² found, while working with rabbits, that gonadectomy combined with adrenalectomy was the most powerful stimulus for thymic regeneration, but thyroid secretion was necessary for this change to take place. Pregnancy is known to hasten thymic involution, and Viets, Schwab and Brazier³ presented a case in which a marked remission of myasthenic symptoms occurred during pregnancy.

In recent years, thymectomy has been resorted to with gratifying results in many of the cases of myasthenia gravis. For this reason a histopathologic study was made of the thymus in this disorder. Since thymic hyperplasia occurs also in other endocrine disorders, a compari-

From the Section on Surgical Pathology (Dr. McDonald) and the Division of Surgery (Drs. Bryan and Clagett), Mayo Clinic.

* Abridgment of a thesis submitted by Dr. Bryan to the Faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Master of Science in Surgery.

1. Simpson, M. E.; Li, C. H.; Reinhart, W. O., and Evans, H. M.: *Proc. Soc. Exper. Biol. & Med.* **54**:135, 1943.

2. Marine, D.; Manley, O. T., and Baumann, E. J.: *J. Exper. Med.* **40**:429, 1924.

3. Viets, H. R.; Schwab, R. S., and Brazier, M. B., cited by Wilson, A., and Stoner, H. B.: *Quart. J. Med.*, **13**:1, 1944.

son was made with the thymic hyperplasia of hyperthyroidism. The thymuses of hyperthyroid patients were selected for the comparative study because of the availability of specimens. The cases were limited to those of exophthalmic goiter because it was thought that the pathologic features would be more constant.

Cancers of the thymus were not included, since a report on such tumors was made previously by Murray and one of us.⁴ Also, extensive investigation of the complex endocrinologic problems of the thymus is outside the scope of this paper.

REVIEW OF THE LITERATURE

Normally the thymus begins to involute at puberty. The involution probably is due to the initiation of gonadal function. Also at puberty there is involution of the lymph nodes.⁵ Hammar⁶ showed that from birth until puberty there is an absolute increase in the weight of the thymus, and that after puberty there is involution without complete disappearance. However, Kasarinow⁷ stated that there is little if any growth after birth.

Reinhart and Wainman⁸ studied the thymus, the lymph nodes and the spleen in male rats after thyroidectomy, castration and replacement therapy and observed that when changes occur in one of these lymphoid tissues they are likely to occur in the others.

In myasthenia gravis, the myoneural junction is affected, and there is manifested an abnormal fatigability of the skeletal muscles with slow progression and remission. The muscles most frequently affected are those supplied by the cranial nerves. It was Weigert⁹ who, in 1901, first demonstrated persistence of the thymus in myasthenia gravis. Bell,¹⁰ in 1917, reported that in 27 of 56 cases of myasthenia gravis thymic abnormality occurred. Blalock and co-workers¹¹ summarized the literature on the thymus in myasthenia gravis up to 1939 when they presented data on 110 patients who had come to operation or autopsy; of 53 of these patients who had some abnormality, 31 had tumors and 22 had enlarged or persistent thymuses.

4. Murray, N. A., and McDonald, J. R.: *Am. J. Clin. Path.* **15**:87, 1945.

5. Cowdry, E. V.: *Special Cytology: The Form and Functions of the Cell in Health and Disease*, ed. 2, New York, Paul B. Hoeber, Inc., 1932, vol. 2, p. 836.

6. Hammar, J. A.: *Klin. Wchnschr.* **8**:1385, 1929.

7. Kasarinow, G., cited by Bratton, A. B.: *J. Path. & Bact.* **28**:609, 1925.

8. Reinhart, W. O., and Wainman, P.: *Proc. Soc. Exper. Biol. & Med.* **49**:257, 1942.

9. Weigert, C., cited by Murray and McDonald.⁴

10. Bell, E. T.: *J. Nerv. & Ment. Dis.* **45**:130, 1917.

11. Blalock, A.; Mason, M. F.; Morgan, H. J., and Riven, S. S.: *Ann. Surg.* **110**:544, 1939.

Exophthalmic goiter was shown by Markham,¹² in 1858, to be associated with an enlarged thymus. Other early investigators presented similar reports.¹³ Kocher,¹⁴ in 1914, published data on 14 cases of exophthalmic goiter, in 42 per cent of which hyperplasia of the thymus and of other lymphoid organs occurred. Margolis¹⁵ stated that the incidence of thymic hyperplasia in cases of exophthalmic goiter is 85 per cent.

There seems to be a reciprocal effect between the thyroid gland and the thymus, the former stimulating, the latter inhibiting, the production of estrogen and androgen. Gudernatsch¹⁶ found that the thyroid gland stimulates, and the thymus suppresses, growth and differentiation of tissues. In one of McEachern's¹⁷ cases of myasthenia gravis combined with hyperthyroidism there was marked aggravation of myasthenic signs and symptoms several days after thyroidectomy. On the other hand, Kowallis and co-workers¹⁸ reported a case in which the myasthenic and hyperthyroid manifestations disappeared after subtotal thyroidectomy.

EMBRYOLOGIC FEATURES

The thymus is of ectodermal-endodermal origin, the ectoderm of the cervical sinus joining the primitive cortex of the gland by migrating to surround the endodermal thymus which is derived from the third branchial pouch. At the 24 mm. stage there are, according to Norris,¹⁹ three zones: (1) the outer cortical layer of ectoderm, (2) the intermediate lymphocytic layer, which is of mesenchymal origin and which secondarily invades the gland after the entrance of the vessels and connective tissue, and (3) the inner medullary endodermal component. The two types of reticulum cells are (1) the compact epithelial endodermal mass which forms the syncytial cytotreticulum and (2) the ordinary fibrous reticulum of the gland which is derived from the cells of the adventitia of the small blood vessels, the capsule and the interlobular septums.

Hassall's corpuscles are formed from some of the cells which are crowded into the medulla by the subcortical lymphocytes. The early

12. Markham, cited by Kowallis, Haines and Pemberton.¹⁸

13. MacKenzie, H., and Edmunds, W.: *Tr. Path. Soc. London* **48**:192, 1897.
Soupault, M.: *Bull. Soc. anat. de Paris* **72**:592, 1897.

14. Kocher, A.: *Arch. f. klin. Chir.* **105**:924, 1914.

15. Margolis, H. M.: *Ann. Int. Med.* **4**:1112, 1931.

16. Gudernatsch, cited by Capelle, W.: *Deutsche Ztschr. f. Chir.* **235**:280, 1932.

17. McEachern, D.: *Medicine* **22**:1, 1943.

18. Kowallis, G. F.; Haines, S. F., and Pemberton, J. deJ.: *Arch. Int. Med.* **69**:41, 1942.

19. Norris, E. H.: *Contrib. to Embryol.* **27**:193, 1938.

Hassall's corpuscles are seen to be made up of a plaquelike arrangement of two to three of these large cells having large ovoid vesicular nuclei.

Thus, the endoderm of the third branchial pouch gives rise to the syncytial cytotreticulum which is apart from the fibrous reticulum, the lymphocytes invade the gland from without and have a mesenchymal origin, and the ectodermal cervical sinus gives rise to the primitive thymic cortex and the corpuscles of Hassall.

HISTOLOGIC CHARACTERISTICS

Maximow and Bloom²⁰ described the thymus as consisting of two lobes made up of many lobules which have a light-staining medulla surrounded by a darker-staining cortical portion. In the cortex are densely packed masses of small cells which are morphologically identical with the small lymphocyte. These cells average 6 microns in diameter and have a very small amount of cytoplasm, a dark nucleus with heavy chromatin particles, and a distinct nucleolus. Among these are elongated reticular cells with a pale round or oval nucleus, a smooth nuclear membrane, a few small chromatin particles and one or two nucleoli. As the examination proceeds from the cortex to the medulla, one notes that most of the lymphocytes disappear abruptly.

The medulla consists predominantly of reticular cells, which can be seen to form a syncytium, the meshes of which are filled with lymphocytes. Cowdry²¹ said that the reticular cell of the thymus looked to him exactly like the reticular cell of the lymph nodes and the spleen. The medulla also contains Hassall's corpuscles, which are acidophilic, rounded structures 30 to 100 microns in diameter composed of concentrically arranged cells, many of which show evidence of degeneration and hyalinization. Reticular cells are connected at one or more places at the periphery of Hassall's corpuscles.

PATHOLOGIC FEATURES

Myasthenia Gravis.—There are no constant pathologic changes in the thymuses of patients with myasthenia gravis; when thymoma is excluded, there remain only normal glands or those which have become hyperplastic. The "persistent" glands are considered to be those glands of older age groups which have not atrophied and which have not been replaced by fat. These same persistent glands should be considered normal, since there is a great variance in the preservation of the normal appearance of the thymus, and they are not hyperplastic nor are they cancerous. We therefore have referred to all the thymuses which are

20. Maximow, A. A., and Bloom, W.: A Textbook of Histology, ed. 4, Philadelphia, W. B. Saunders Company, 1942, p. 324.

21. Cowdry, E. V.: A Textbook of Histology: Functional Significance of Cells and Intercellular Substances, ed. 2, Philadelphia, Lea & Febiger, 1938, p. 162.

not cancerous or hyperplastic as normal glands whether much or little of the glandular tissue remained.

There are two types of hyperplasia of the thymus: that of the epithelial syncytium and that of the lymphocytic tissue. Norris²² stated that in myasthenia gravis all pathologic changes of the thymus, cancer excluded, represent a varying degree of epithelial hyperplasia, the benign tumor being interpreted as representing extreme epithelial hyperplasia. Homburger²³ found extreme epithelial metaplasia in 2 cases of myasthenia gravis and moderate epithelial metaplasia in 3 cases of thyrotoxicosis and 2 cases of incidentally observed persistence of the thymus in more than 6,000 autopsies. Hoxie²⁴ spoke of the predominance of epithelial elements in myasthenia gravis. Bell¹⁰ and Miller²⁵ also stated that in myasthenia the epithelial elements predominate. Likewise, Hardymon and Bradshaw²⁶ reported epithelial hyperplasia in a case of myasthenia gravis.

Sloan,²⁷ however, in reexamining Miller's slides, found no hyperplasia of the epithelium but did find definite lymph follicles. Sloan examined the thymuses of 150 people who died suddenly without previous chronic disease, in 14 of which he found lymph follicles with germinal centers; however, when the follicles occurred in normal glands they were few. He found occasional lymph follicles in most normal thymus glands when serial sections were examined. Blalock²⁸ reported 10 cases of lymphoid hyperplasia with germinal center formation, in one of which the hyperplasia was impressive, in a series of 20 cases in which thymectomy had been performed for myasthenia. Also, there were 2 cases of tumor in this group. Giordano and Haymond²⁹ found diffusely scattered homogeneous lymphocytes in a reticulum cell stroma.

Norris¹⁹ considered lymphorrhages of skeletal muscle pathognomonic of myasthenia, but Giordano and Haymond found lymphorrhages in only two thirds of their cases, and Dudgeon and Urquhart³⁰ found lymphorrhages in 8 of 9 cases of exophthalmic goiter and also in cases of myositis.

Hyperthyroidism.—As Margolis pointed out in his work on thymic hyperplasia in hyperthyroidism, there are relatively few reports of a careful histologic study of the thymus. If the weight of the thymus

22. Norris, E. H.: *Am. J. Cancer* **27**:421, 1936.

23. Homburger, F.: *Arch. Path.* **36**:371, 1943.

24. Hoxie, G. H.: *J. Missouri M. A.* **14**:389, 1917.

25. Miller, H. G.: *Arch. Path.* **29**:212, 1940.

26. Hardymon, P. B., and Bradshaw, H. H.: *Surg., Gynec. & Obst.* **78**:402, 1944.

27. Sloan, H. E.: *Surgery* **13**:154, 1943.

28. Blalock, A.: *J. Thoracic Surg.* **3**:316, 1944.

29. Giordano, A. S., and Haymond, J. L.: *Am. J. Clin. Path.* **14**:253, 1944.

30. Dudgeon, L. S., and Urquhart, A. L.: *Brain* **49**:182, 1926.

exceeded the normal, the gland was designated as hyperplastic. Abnormal weight, however, cannot be used as the criteria of hyperplasia. Regrettably, Margolis did not distinguish between lymphoid and epithelial hyperplasia in his series. He reported that there was an 85 per cent incidence of thymic hyperplasia in his cases of exophthalmic goiter.

In 6,000 autopsies Homburger found 3 instances of thyrotoxicosis in which moderate epithelial hyperplasia of the thymus, with numerous young and hyalinized Hassall's corpuscles, had occurred. In 2 cases the separation of cortex and medulla was distinct. In 2 cases the interstitial tissue was fibrous-adipose, while in the third it was adipose.

THE PRESENT STUDY: MATERIALS AND METHODS

All the definitely proved cases of myasthenia gravis in which autopsy or thymectomy had been performed at the Mayo Clinic, with the exception of the cases of thymoma, were included in this study. Dr. L. M. Eaton, of the department of neurology and psychiatry, selected the cases of myasthenia gravis, in all of which, he thought, on the basis of clinical findings and the results of therapeutic trials of prostigmine, the criteria of the disease were fulfilled. When the tissue had been examined histologically and thymoma had been ruled out, 23 cases remained.

For purposes of comparison, a study was made of 20 cases of exophthalmic goiter, taken at random, in which a diagnosis of parenchymatous hypertrophy had been made on the surgical specimen removed at thyroidectomy and in which the patient had died within a brief period after operation and had come to autopsy.

The data on the weight, the size and the gross appearance of the specimen used were taken from the pathologist's report, from the autopsy protocols, from the surgical records or from a combination of these. This was done to obtain a much more accurate and precise description than could be obtained by examination of the fixed specimens alone. In many cases such data were unavailable. Also, slides of the spleen and of lymph nodes, when available, were included in this study. Hematoxylin and eosin stains were used routinely except in 1 instance in which sudan III was used.

It is most essential, in the consideration of this subject, to realize what the term "hyperplasia" implies. According to MacCallum,³¹ hyperplasia of an organ is the result of an increase of the number of its cells, whereas hypertrophy is the result of an increase in the size of its elements. Not infrequently, both processes are in evidence. In essential agreement with these definitions are those of Smith and Gault,³² Boyd,³³ Duval and Schattenberg³⁴ and Wood.³⁵

31. MacCallum, W. G.: *A Textbook of Pathology*, ed. 7, Philadelphia, W. B. Saunders Company, 1940, p. 76.

32. Smith, L. W., and Gault, E. S.: *Essentials of Pathology*, New York, D. Appleton-Century Company, Inc., 1938, p. 35.

33. Boyd, Wm.: *Textbook of Pathology*, ed. 4, Philadelphia, Lea & Febiger, 1943, p. 254.

34. Duval, C. W., and Schattenberg, H. J.: *Textbook of Pathology: A Correlation of Clinical Observations and Pathological Findings*, ed. 1, New York, D. Appleton-Century Company, Inc., 1939, p. 98.

35. Wood, F. C.: *Textbook of Pathology*, ed. 16, Baltimore, William Wood & Company, 1936, p. 77.

Therefore, in order to interpret the microscopic observations properly, it was necessary to go over many slides of thymuses, including fetal thymuses, normal thymuses of different age groups (fig. 1) and also thymomas. Even after this had been done, the interpretation of the observations was often difficult.

In all cases the criterion which had to be met to allow a possible diagnosis of hyperplasia was an increase in the density or the number of the essential elements of the gland. Many of the specimens examined showed an increase in the density or the number of the essential elements, but the content of thymic tissue in the different sections was often only 5 or 10 per cent; obviously, with thymic cells constituting such a small percentage, there was no hyperplasia. The density of cells

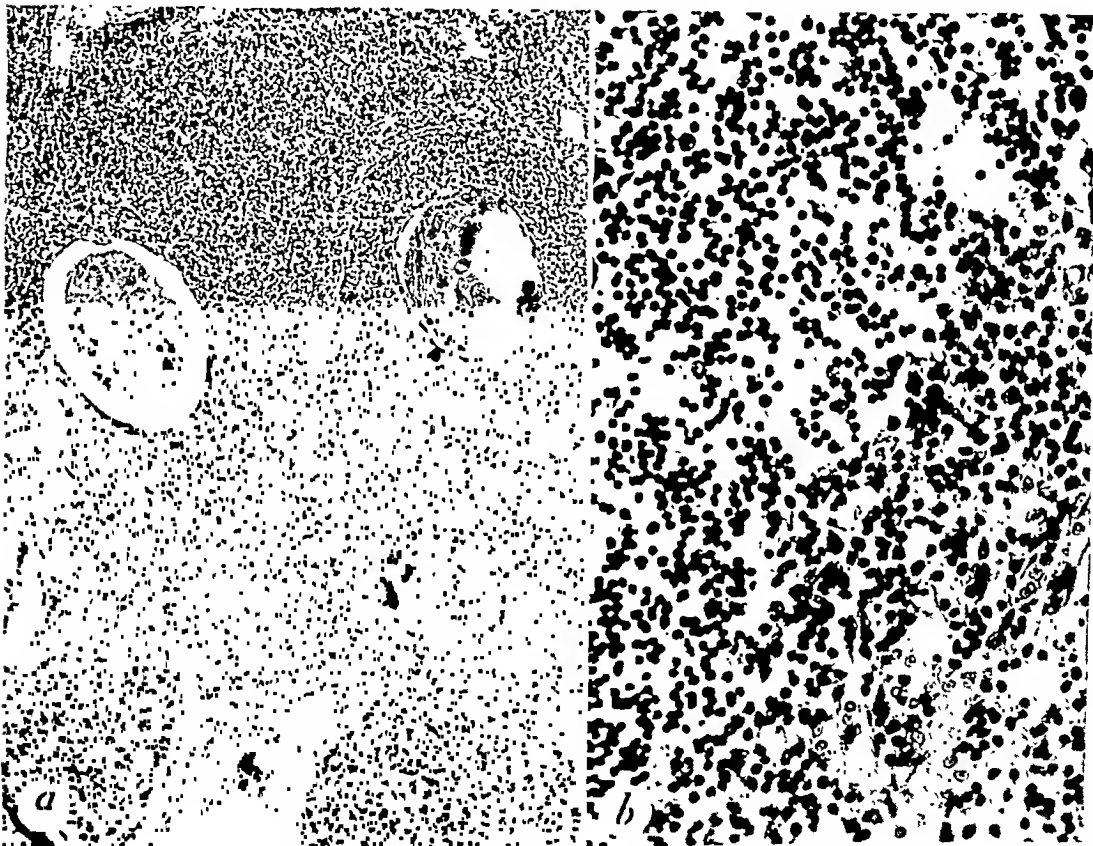


Fig. 1.—Normal thymus: (a) $\times 60$; (b) $\times 330$.

varied from that of no increase over the normal to that of a marked increase. Associated with the marked increase in density were other indications of potential, if not actual, activity, including the presence of active, proliferative germinal centers.

An attempt was made to grade the extent of hyperplasia of any given section as seen under the microscope. Originally, the classification was based on three grades: minimal, moderate and marked hyperplasia; this, however, was soon found to be too fine a differentiation. Therefore, we patterned our nomenclature after that of Norris¹⁹ and classified the hyperplasia microscopically as moderate and extreme. Hyperplasia was considered moderate when the density of essential thymic elements noticeably exceeded normal, and it was considered extreme when

there was a striking increase over normal. However, the hyperplastic thymic glands in this series showed lymphocytic hyperplasia, whereas those in Norris' series showed hyperplasia of the epithelial elements. In only the myasthenia group were there active germinal centers, and these appeared to be overactive in the cases of extreme hyperplasia. Also, the type of hyperplasia, whether lymphoid or epithelial, was noted.

Another factor, as previously mentioned, is the amount of the gland which is actually thymic tissue, the connective tissue elements, such as fibrous and adipose tissue, being excluded. In this study the percentage of thymic tissue was estimated in regard to the whole gland after a study of several sections of the gland had been made. It was observed that in the glands in which hyperplasia was obvious the percentage of thymic tissue was always at least 70. Therefore, we arbitrarily assumed that in the adult age group a gland, to fulfil another criterion for hyperplasia, should be composed in at least 70 per cent of essential thymic elements.

The age of the patient was another factor that entered into the interpretation, because it is well known that the appearance of the gland in a child is not like that in the older age groups. A normal thymus of a child shows, histologically, a greater percentage of essential thymic elements and increased density. Therefore, in the younger age groups a gland, to be classified as hyperplastic, had to have a correspondingly greater percentage of essential thymic elements than the 70 per cent minimum established for adults.

The glands whose sections did not fulfil the foregoing criteria were then designated as normal glands or thymic fat; the latter designation was used when the thymic tissue was so minimal in the connective tissue that identification of it was next to impossible. The thymus was labeled "normal" when the lobules were distinct, when identification of tissue could be made with ease and when there was no increase in the number of essential thymic elements or when the percentage of thymic tissue was too low for the gland to be considered as hyperplastic.

It may be possible to designate some of these tissues as hyperplastic on the basis of microscopic appearance alone; however, if this is done, one important procedure has been omitted, namely, the determination of gross hyperplasia based on the weight of the gland and the percentage of thymic tissue. Hammar⁶ published data on the weights of thymuses of all age groups. Furthermore, he estimated the relative proportions of parenchymatous and interstitial tissues by a more reliable method than that of microscopic estimation. This method consists in tracing the outlines of these tissues in magnified sections and measuring the respective areas with the planimeter. We have taken these values as the standard for comparison; also, we have used Hammar's values for the weight of the thymus. The weights at different ages varied somewhat from series to series, but there was no significant difference between the sexes; therefore, the weights used were the combined averages for the two sexes.

Young and Turnbull³⁶ stated that their microscopic estimates of the proportions of glandular and interstitial tissues and those of Bratton³⁷ differ significantly from the values derived by the planimetric method of Hammar,³⁸ but it seems to us that there is no other practical way to estimate percentages than by simple microscopic study. They also pointed out that naked eye estimation of the ratio of glandular to connective tissue is valueless.

36. Young, M., and Turnbull, H. M.: *J. Path. & Bact.* **34**:213, 1931.

37. Bratton, A. B., cited by Young and Turnbull.³⁶

38. Hammar, J. A., cited by Young and Turnbull.³⁶

Therefore, if we know the weight of the gland and the percentage of the glandular tissue, then by simple multiplication we can determine the weight of the glandular tissue. This value can be compared with normal values, and if it exceeds the upper limit of normal, there is an increase in the number of essential thymic elements, a condition which, by definition, is hyperplasia (table 1). The expected average weight of glandular tissue in a series of normal glands is determined by multiplying the average weight of the glands by the average percentage of glandular tissue. The estimated upper limit of normal is derived, for all practical purposes, by multiplying the average weight of the glands plus twice the standard deviation by the average percentage of glandular tissue. These values have been plotted, and a curve has been drawn which will give the estimated amounts of glandular tissue for the different age groups (fig. 2). If the weight of the gland in question exceeds the estimated upper limit of normal, then for all

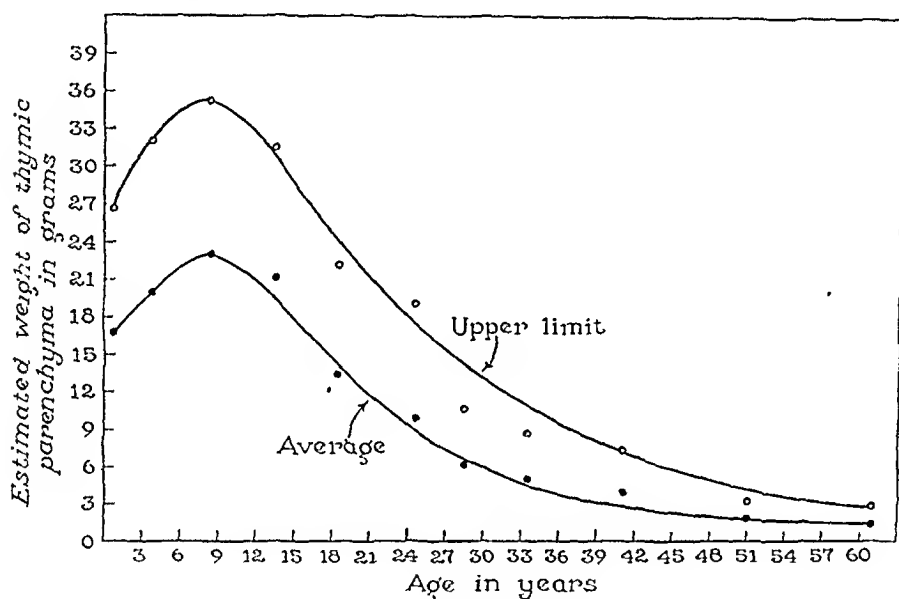


Fig. 2.—Graphically fitted trend lines of estimated average normal and maximal normal weights of thymic parenchyma derived from data in table 1.

TABLE 1.—Estimated Average Normal and Maximal Normal Weights of Thymic Parenchyma Based on Hanmar's Data

Age, Yr.	Thymus				Thymic Parenchyma	
	Average Weight, Gm.	Standard Deviation, Gm.	Average Weight +2 Times Standard Deviation, Gm.	Thymic Tissue, Average Percentage	Estimated Average Normal Weight, Gm.	Estimated Maximal Normal Weight, Gm.
0-1.....	20.5	6.1	32.7	81	16.6	26.5
1-6.....	25.2	8.1	41.4	78	19.7	32.3
6-11.....	30.4	8.4	47.2	75	22.8	35.4
11-16.....	29.1	7.0	43.1	73	21.2	31.5
16-21.....	24.9	8.1	41.1	54	13.4	22.2
21-26.....	20.3	9.2	38.7	49	9.9	19.0
26-31.....	18.1	6.5	31.1	34	6.2	10.6
31-36.....	19.6	7.5	34.6	25	4.9	8.7
36-46.....	18.9	7.4	33.7	22	4.2	7.4
46-56.....	16.1	10.8	37.7	11	1.8	4.1
56-66.....	14.3	7.5	29.3	10	1.4	2.9
66 and over.....	12.3	5.9	24.1	9	1.0	2.2

practical purposes and by definition hyperplasia has occurred. The peak of the curve representing the upper limit of normal is at 9 years. Table 2, derived from data in figure 2, gives the estimated values for the average normal and the maximal normal weights of thymic parenchyma at stated ages and can be used as a simple guide in determining whether a particular gland is grossly hyperplastic.

FINDINGS

Myasthenia Gravis.—Grossly, the thymic gland was found to be apparently hyperplastic, with the typical butterfly pattern, in 16 of the 23 cases. As to the remaining 7 cases, a small amount of thymic tissue was observed in 1, the thymic tissue had been replaced by fat in 4 and no gross description was available in 2. Microscopically, there was extreme lymphoid hyperplasia in 4 cases (fig. 3) and moderate lymphoid hyperplasia in 2. In the remaining 17 cases the thymus was considered normal or had been replaced by fat. There were no examples of epithelial hyperplasia.

TABLE 2.—*Estimated Average Normal and Maximal Normal Weights of Thymic Parenchyma at Stated Ages as Derived from the Fitted Lines of Figure 2*

Age, Yr.	Estimated Weight of Thymic Parenchyma, Gm.	
	Average Normal	Maximal Normal
1.....	17.2	27.7
5.....	21.4	34.0
10.....	22.4	34.8
15.....	18.5	29.0
20.....	13.6	21.9
25.....	9.1	16.0
30.....	6.1	11.9
35.....	4.2	9.3
40.....	2.9	7.4
45.....	2.2	5.7
50.....	2.0	4.4
55.....	1.8	3.5
Over 55.....	1.5	2.9

The cytotreticulum of the thymus was arranged, in 15 of the 23 cases, as single cells or groups of two or three cells, while in 4 cases only single cells or occasional small groups of cells were observed. In 2 cases in which the thymus had been replaced by fat, no syncytial cells were found. In 2 cases there was a clustering of the syncytial cells, but only in isolated areas, and in neither instance was it more than might be seen in a normal gland.

The frequency of occurrence and the age of Hassall's corpuscles varied tremendously. In 7 of the 23 cases the Hassall's corpuscles observed were mainly young ones; in the others chiefly hyalinized or calcified corpuscles were present. In 14 of the 23 cases only occasional Hassall's corpuscles were noted, while in 8 cases there was evidence of moderate or frequent occurrence; in 1 case, in which the thymic tissue had been replaced by fat, none were found. The presence of Hassall's corpuscles of a uniform age was not characteristic of any of the types of cases. However, the hyperplastic glands had a tendency to have a larger number of corpuscles.

The interlobular trabeculae were adipose in 15 cases, fibrous in only 2 cases and fibrous-adipose in 6 cases. There was a tendency in the cases of hyperplasia

for only small amounts of interlobular connective tissue to occur, but this tissue, it was observed, could be any one of the three varieties mentioned in the preceding sentence.

Calcium was found in only 5 cases. It usually was seen in Hassall's corpuscles, although it was noted once in the arterioles and once in a gland which had been replaced by fat. Calcium was found in only 1 case of extreme hyperplasia and then only in the corpuscles of Hassall. In all the hyperplastic glands there was distinct lobulation and in 6 normal glands quite definitely outlined lobules. The differentiation of cortex and medulla was clearcut in 2 hyperplastic glands, only fairly well marked in 3 and indistinct in 1. In the 17 normal glands there was

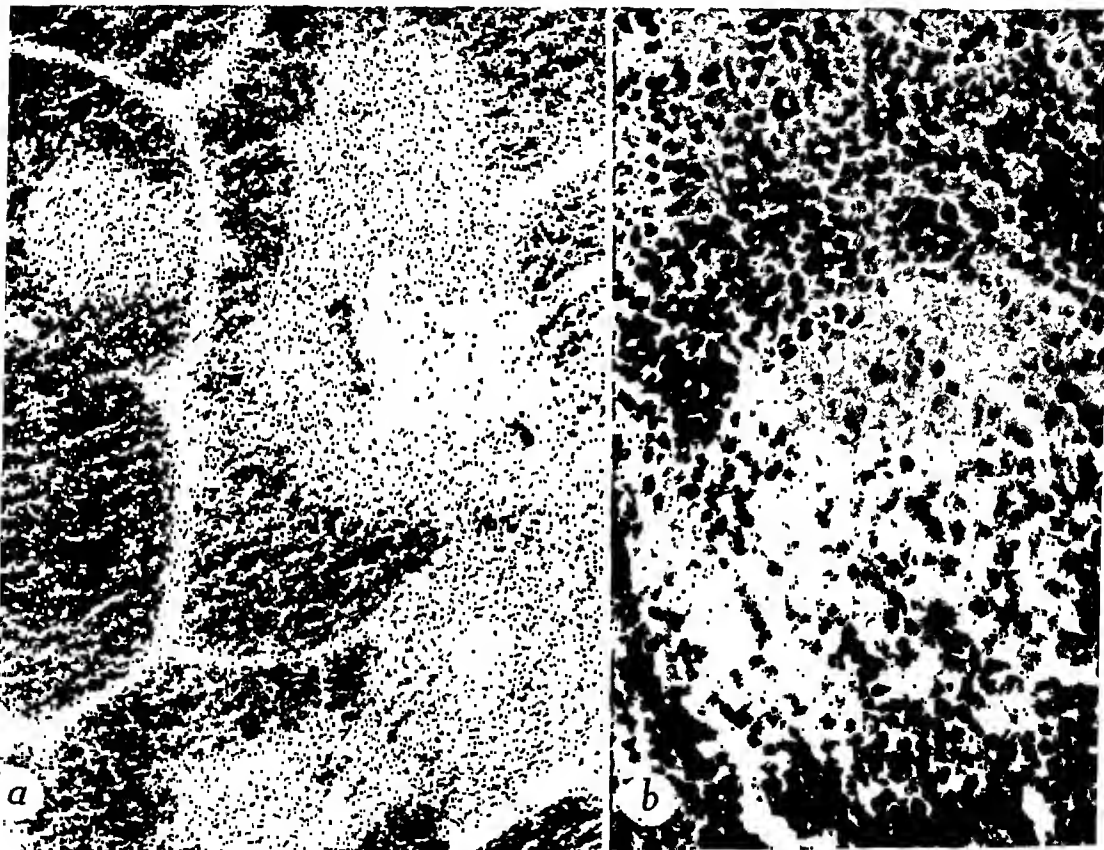


Fig. 3.—Extreme lymphoid hyperplasia of the thymus in myasthenia gravis: (a) $\times 60$; (b) $\times 330$.

definite differentiation of cortex and medulla in 2, moderate differentiation in 4 and no differentiation in 11.

In the 6 glands of the myasthenia series which were subsequently designated as hyperplastic, the lymphoid tissue was definitely increased over normal, and the areas of extreme hyperplasia were dense and gave an impression of being functionally quite active, with many germinal centers. There were germinal centers along with increased density in the cases of moderate hyperplasia, but this feature was not as characteristic as in the extreme group.

Exophthalmic Goiter.—All the specimens of thymuses in this group were obtained at necropsies. In 8 of the 20 cases of exophthalmic goiter there was no

available gross description. Six of the glands were replaced by fat, and 2 were normal in appearance. In 4 of the 20 cases there was apparent enlargement of the thymus; in one of these the gland exhibited moderate lymphoid hyperplasia microscopically and was described as being large and fleshy and as weighing 39 Gm. (fig. 4). Since this weight was six times the normal expected weight, and since approximately 90 per cent of the tissue was essential thymic tissue, it was evident that this gland was hyperplastic grossly and microscopically. The second of the 4 apparently enlarged glands was described as being enlarged but of normal appearance, with a weight nine times normal, but microscopically this gland was shown

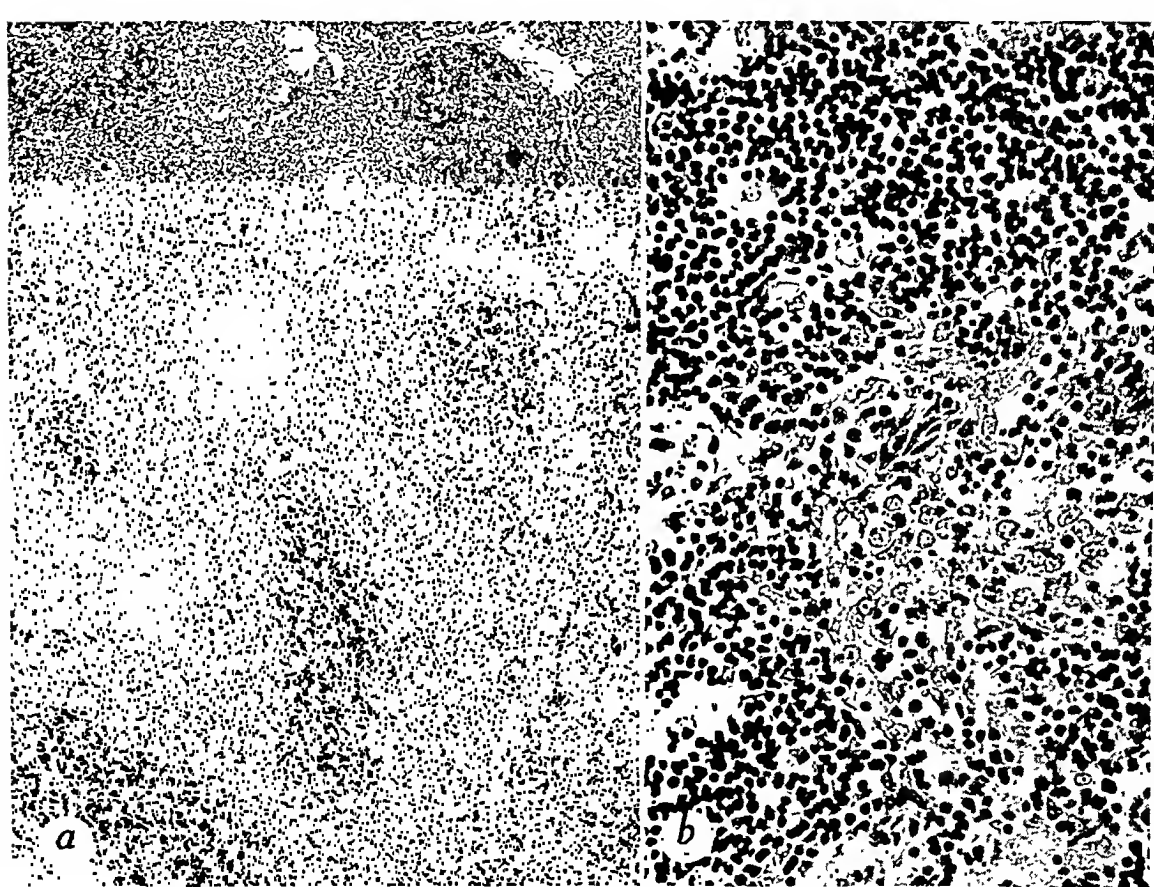


Fig. 4.—Moderate lymphoid hyperplasia of the thymus in exophthalmic goiter: (a) $\times 60$; (b) $\times 330$.

to be normal. The third apparently enlarged thymus was reported as being markedly "persistent," but the weight was unobtainable, and microscopically this gland, too, appeared to be normal. The fourth apparently enlarged thymus, which was described as being hypertrophied, was an excellent example of the error of designating a gland as hyperplastic on the basis of weight alone. In this case the weight of the thymus of a patient 41 years old was 35 Gm., which is approximately five times the upper limit of normal, but microscopically only 1 or 2 per cent of the gland was composed of essential thymic elements, the remainder being fat. Obviously, this could not have been a hyperplastic gland.

In the exophthalmic goiter series the epithelial syncytium of the thymus did not vary greatly from that in the myasthenia series. In 10 cases the cytoreticulum occurred as single cells or as groups of two or three cells. In 3 cases the syncytial elements ranged from clusters of cells down to single cells. In 4 cases there were next to no epithelial elements, and in 1 case none were seen; in these cases the glandular tissue was minimal, most of the thymic tissue having been replaced by fat.

In only 2 cases were young Hassall's corpuscles in the majority, but in 9 other cases young corpuscles were intermingled with hyaline and calcified corpuscles. In 9 cases hyaline or calcified corpuscles alone occurred. The age of the patient apparently had little to do with the type of corpuscle found, since the average ages of those with and those without young corpuscles were almost exactly the same. The age of the patient had little to do with the age of the corpuscles, since young corpuscles were seen in a normal thymus of a 72 year old man. In 15 glands frequent occurrence of corpuscles was noted, while in 4 Hassall's corpuscles were noted only occasionally, and in 1 there were no corpuscles since the gland was replaced by fat. In 15 of the 20 cases of the exophthalmic goiter series Hassall's corpuscles occurred frequently, the average age of the patients being 48 years; on the other hand, in only 8 of the 23 cases of the myasthenia gravis series did Hassall's corpuscles occur frequently, the average age of the patients being 37 years. The ages ranged from 4 to 72 years in the hyperthyroidism series and from 15 to 74 years in the myasthenia series.

The characteristics of the interlobular trabeculae were almost identical in the exophthalmic goiter series and the myasthenia series, the trabeculae in the former being adipose in 13 instances, fibrous in 2 and fibrous-adipose in 5. Again, in the gland that was hyperplastic there was only a small amount of fibrous-adipose tissue. In the normal thymus of an 11 year old girl there was a small amount of fibrous tissue in the trabeculae.

In 6 cases calcium was found in some of the Hassall's corpuscles, whereas in 14 cases no calcium was found.

There was distinct lobulation of the thymus in 17 of the 20 cases, but in 3 cases in which little thymic tissue was present there was no lobulation but merely a few nests of thymic tissue scattered through the fat. Differentiation of cortex and medulla was fairly definite in only 4 glands, including the single hyperplastic gland, but in the remaining 16 glands there was no differentiation.

In only 1 gland was the lymphoid tissue hyperplastic, and this hyperplasia was only moderate, without germinal centers. In other glands varying amounts of lymphoid tissue were observed, but none had a greater amount than can be seen in a series of normal thymuses. In none of the 20 glands in the hyperthyroidism series were any active germinal centers found.

COMMENT

Thymic Hyperplasia.—In the series of 23 cases of proved myasthenia gravis, exclusive of thymoma, in which thymectomy had been done at the clinic up to April 1946, extreme thymic hyperplasia of the lymphoid type was evident microscopically in 4 cases, moderate lymphoid hyperplasia occurred in 2 and the gland was either normal or consisted of thymic fat in 17. Of the 17 normal glands, 12 showed the density of cells definitely increased as compared with the normal density, and 2 exhibited a few areas in which there was clustering of the epithelial

elements; however, since the average content of thymic tissue in the sections of these 2 glands was only 29 per cent, the glands obviously could not be classified as hyperplastic.

Using the same microscopic criteria as in the myasthenia series, we found no extreme hyperplasia of the thymus in the 20 cases of exophthalmic goiter, and moderate lymphoid hyperplasia in only 1 instance. There was 1 case in which the epithelial elements were increased over the normal, but the other parts of the gland were normal; although 80 per cent of the specimen was thymic tissue, this gland was called normal. Two other glands exhibited a few areas of epithelial clustering, but the percentage of thymic tissue was far below the arbitrary minimum. Five glands showed increased density of cells in the various fields of the slide, but the percentage of thymic tissue was too low to permit considering them as hyperplastic. The average content of thymic tissue in the 8 glands in which density of cells was definitely increased but below the 70 per cent minimum was 38 per cent.

Using Hammar's data, we found, among 13 cases of myasthenia gravis in which the weight of the thymus was available, 2 cases in which the weight exceeded significantly the estimated upper limit of normal, 5 cases in which it exceeded the average weight but was still in the normal range, and 6 cases in which it was less than the average. Comparing the weights obtained by the two methods, we found that, of the glands which showed microscopically extreme hyperplasia, only 1 actually had more thymic tissue than normal, whereas 2 had even less than the average. Of the 2 glands with moderate hyperplasia, the weight of glandular tissue was increased in one, and it was greater than average but within the normal range in the other. In no case did the weight of a gland which was normal microscopically exceed the upper limit of normal.

In the exophthalmic goiter series there were 9 cases in which the weights were available. In 7 of these, significantly excessive amounts of glandular tissue were present, and the glands were, therefore, hyperplastic; however, of these 7 cases, in only 1 was microscopic hyperplasia evident; in the others the gland was normal microscopically.

Other lymphoid organs were also studied to see whether there was any concurrent hyperplasia in the spleen and lymph nodes. In 1 case in the myasthenia series, moderate lymphoid hyperplasia of the lymph node studied was observed, but microscopically the thymus was normal in every respect. In another case, a normal thymus with some increased density of the lymphocytes was noted, whereas the spleen exhibited moderate reticuloendothelial hyperplasia. In another case, in which the thymus showed histologically extreme lymphoid hyperplasia, the lymph node from the trachea showed marked reticuloendothelial hyperplasia,

a condition that is, however, consistent with that seen in anthracosis (fig. 5 *a*). In the exophthalmic goiter series there was no associated hyperplasia of the spleen or lymph nodes, except that one lymph node from the lung showed reticuloendothelial hyperplasia due to anthracosis. Thus it may be seen that there was no correlation between hyperplasia of the thymus and hyperplasia of the lymphoid organs in either of these two series. Young and Turnbull showed that there is considerable variability of volumes of lymphoid structures in the different age groups

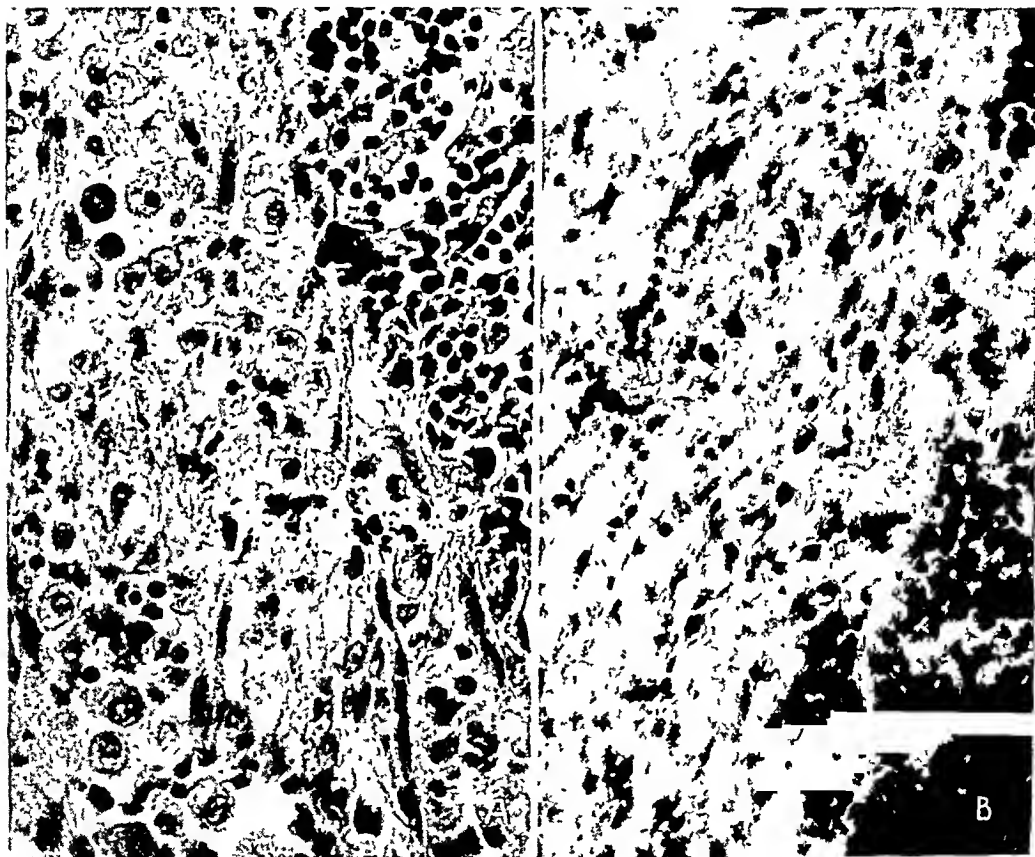


Fig. 5.—(*a*) Reticuloendothelial hyperplasia in a normal lymph node; $\times 350$
(*b*) Fat globules without adult fat cells in thymoma; sudan III; $\times 285$.

but that the interrelationships of the several lymphoid structures in regard to volume are statistically insignificant.

An attempt was made to correlate the clinical findings, the severity and duration of symptoms and the improvement following surgical intervention with the presence of microscopic and gross evidence of hyperplasia, but there was no correlation in this series; consequently it seems that the pathologist cannot, by gross or microscopic examination, either ascertain the clinical course preoperatively or prognosticate the results

of surgical treatment. Keynes³⁹ stated that the prognosis is better in cases in which there is a history of short duration of the condition and poorer in cases in which the disease is of long duration or in which there is a tumor. His colleagues, Collins and Bratton,⁴⁰ have investigated thymus glands from normal adults and adults with myasthenia gravis, and they have found it not easy to detect any constant difference between them; they have expressed the belief that in most myasthenic glands there is an abnormal development of foci of clear cells resembling the so-called germinal centers of lymph nodes.

Thymoma.—It was observed in the preliminary study of the thymoma group that differentiation from extreme hyperplasia is not always easy and that none of the histologic criteria used in diagnosis are constant or limited to cancers or thymoma. Similar kinds and numbers of Hassall's corpuscles, calcium, hyalinized trabeculae, lining up of cells along trabeculae, perivascular grouping, necrosis, foam cells and mitotic figures may be found in fetal, normal and hyperplastic thymuses.

However, one of us (J. R. M.) has shown that fat is rarely found in the thymus afflicted with thymoma. An analogous situation is found in the hyperplastic parathyroid gland and the parathyroid adenoma, the latter rarely containing any adult fat cells. There are a few other constant features of thymoma; namely, it is usually encapsulated, it usually exhibits excessively thickened trabeculae and it usually has distorted thymic structure. In one of the specimens of thymoma, after diligent examination, a few adult fat cells were found, but these may well have represented extracapsular extension of the tumor. A section of thymoma stained with sudan III and showing fat globules but no adult fat cells is illustrated in figure 5 *b*.

A descriptive definition of thymoma might be phrased as follows: Thymoma is a circumscribed tumor of the thymus composed of structurally disarranged thymic elements, often with dense dividing trabeculae, and usually devoid of adult fat cells.

SUMMARY

Among 23 proved cases of myasthenia gravis, exclusive of thymoma, in which thymectomy was done, there were 4 in which extreme lymphoid hyperplasia, and 2 in which moderate lymphoid hyperplasia of the thymus, was observed on microscopic examination. In the remainder of the cases the gland either was normal or had been replaced by fat.

Twelve of the 17 normal glands in the myasthenia series showed areas of hyperplasia, but the percentage of glandular tissue was too

39. Keynes, G.: Brit. J. Surg. **33**:201, 1946.

40. Collins and Bratton, cited by Keynes.³⁹

low to permit their being considered hyperplastic, the average content of thymic parenchyma being only 29 per cent.

In none of the 20 cases of hyperthyroidism due to exophthalmic goiter did extreme hyperplasia of the thymus occur, and in only 1 case did moderate lymphoid hyperplasia occur.

Small areas of epithelial clustering occurred in 2 cases of the myasthenia series and in 3 cases of the hyperthyroidism series, but the glands were essentially normal.

When the actual amounts of the glandular tissue of the thymuses were determined and compared with Hammar's weights and with planimetrically determined percentages of thymic tissue, the weight of the parenchyma of 2 of 13 glands in the myasthenia series significantly exceeded the upper limit of normal, while the weight of the parenchyma of 7 of 9 glands in the hyperthyroidism series exceeded the upper limit of normal.

There was apparently no correlation between the extent of hyperplasia determined microscopically and that which was observed when the actual weight of essential thymic elements exceeded significantly the expected upper limit of normal.

There was no correlation, on the basis of microscopic observations, between the extent of hyperplasia of one lymphoid organ and that of another in this study.

No correlation was found between the clinical and the microscopic observations in myasthenia gravis exclusive of thymoma.

The term "thymoma" might be defined as a circumscribed tumor of the thymus composed of structurally disarranged thymic elements, often with dense dividing trabeculae, and usually devoid of adult fat cells.

TESTICULAR TUMORS

II. Interstitial Cell and Miscellaneous Neoplasms

ROBERT E. SCULLY, M.D.

WALPOLE, MASS.

AND

ASA R. PARHAM, M.D.

BOSTON

IN A PREVIOUS communication¹ a simple classification of testicular neoplasms was proposed, and the commonly occurring seminoma and teratoma were discussed from clinical and pathologic points of view. The present report completes the study of testicular tumors, dealing with the interstitial cell and rare miscellaneous types, which together constitute less than 5 per cent.² A number of important contributions in the literature are reviewed. Four cases collected in a review of the files of the department of pathology of the Peter Bent Brigham Hospital from 1914 to 1947 are reported.

MATERIAL AND METHODS

The material consisted of blocks taken from surgically excised primary tumors and metastatic lesions, fixed in Zenker's acetic acid solution and/or 4 per cent formaldehyde solution, embedded in paraffin and stained with eosin-methylene blue and/or hematoxylin and eosin. Fat and connective tissue stains were done when indicated.

CLASSIFICATION

The classification presented now is based on limited personal experience and an incomplete, albeit extensive, review of reported cases; hence, it is necessarily subject to errors of omission.

1. Arrhenoblastoma
2. Interstitial cell tumor
3. Adrenal cortex rest tumor
4. Tubular "adenoma"

From the Departments of Pathology of the Peter Bent Brigham Hospital and Harvard Medical School and the Department of Surgery of the Peter Bent Brigham Hospital. Dr. Scully's present address is: Pondville Hospital, Walpole, Mass.

1. Scully, R. E., and Parham, A. R.: Arch. Path. 45:581, 1948.

2. Friedman, N. B., and Moore, R. A.: Mil. Surgeon 99:573, 1946.

5. Adult carcinomas
 - (a) Spermatocytic seminoma³
 - (b) Multicystic adenocarcinoma⁴
 - (c) Carcinoma of rete testis
6. Tumors of blood vessel origin
7. Tumors of fibrous tissue origin
8. Lymphosarcoma
9. Metastatic tumors

ARRHENOBLASTOMA

Teilum⁵ has reported 3 tumors occurring in males which were histologically identical with Meyer's intermediate type of ovarian arrhenoblastoma.⁶ One occurred in the testicle of a 53 year old man who complained of gynecomastia and presented a history of left scrotal swelling, diminution of libido and loss of potency. After orchidectomy the mammae decreased greatly in size. The second tumor was situated in the spermatic cord of a 4 year old boy who showed no endocrine disturbances. The third was testicular; clinical data regarding it were not available.

INTERSTITIAL CELL TUMOR

Pathologic Aspects.—The interstitial cell tumor is typically nodular and yellowish or yellowish brown⁷; it may or may not be encapsulated⁸; reported specimens have varied from less than 2 cm. in diameter to the dimensions of a child's head.⁸

Microscopically, the large polyhedral cells grow in sheets, cords and lobules, separated by varying amounts of collagenous stroma (fig. 1 *A*); at times columns of cells lie in intimate relation to thin-walled vascular sinusoids, creating a liver-like appearance. The cytoplasm of the neoplastic cells is voluminous and eosinophilic (fig. 1 *B*); it may be loaded with fine fat droplets, or the latter may be dispersed in the form of peripheral halos. Intracytoplasmic lipofuchsin granules are commonly present; Reinke's crystalloids, the characteristic inclusions of the normal interstitial cell, rarely.⁹ The neoplastic nuclei are round, oval or wrin-

3. Masson, P.: *Rev. canad. de biol.* **5**:361, 1946.

4. (a) Stevens, A. R., and Ewing, J.: *Ann. Surg.* **88**:1074, 1928. (b) Stofer, B. E.: *Arch. Path.* **40**:68, 1945.

5. Teilum, G.: (a) *Acta obst. et gynec. Scandinav.* **24**:480, 1944; (b) *Acta path. et microbiol. Scandinav.* **23**:252, 1946.

6. Meyer, R.: *Am. J. Obst. & Gynec.* **22**:697, 1931.

7. Nation, E. F.; Edmondson, H. A., and Hammack, R. W.: *Arch. Surg.* **48**:415, 1944.

8. Warren, S., and Olshausen, K. W.: *Am. J. Path.* **19**:307, 1943.

9. Friedman, N. B., and Ash, J. E.: *Atlas of Genitourinary Pathology Prepared at the Army Institute of Pathology, Washington, D. C., Government Printing Office, 1946.*

kled and contain one or more prominent nucleoli. Mitoses are generally absent but may be abundant.⁷

Rarely the interstitial cell tumor gives rise to metastases; moreover, the latter may occur years after removal of a primary tumor which appeared histologically benign.¹⁰

Endocrinologic Aspects.—A recent collective review of 26 cases by Nation and co-workers⁷ reveals that in 9 cases the tumor was associated with clinical evidence of hormone imbalance. All 6 children in the series showed precocious bodily and sexual development; gynecomastia¹¹ was present in 1. Orchidectomy was followed by partial or complete regression of symptoms in 4. Gynecomastia occurred in 3 of 20 adults; in 2 it regressed postoperatively. One adult experienced a decrease in libido, which returned to normal after orchidectomy.¹² No correlation was found between the duration of the tumor and the postoperative regression of the hormonal changes.

Reports of assays of hormones have been few. In a case of cancerous interstitial cell tumor reported by Masson¹⁰ and Venning¹³ there was slight elevation of urinary gonadotropin and estrogens, and a marked rise in 17-ketosteroids. In the case to be reported, no endocrine changes were evident clinically, nor were assays of hormones made.

Clinical Aspects.—In the large series of Friedman and Moore² the interstitial cell growths constituted 1 per cent of the testicular tumors. Persons of all ages are affected; in adults the maximum incidence is between the ages of 30 and 45,⁷ while in children the onset tends to occur during the fourth or the fifth year of life.⁷

Metastases have been reported in 3 cases,⁸ in one appearing four,¹⁴ and in another nine years,¹⁰ after removal of the primary tumor. The interstitial cell tumor to be reported occurred in a 55 year old man, who was free of metastases ten years seven months after the operation.

CASE 1.—H. M., a 55 year old white man, complained of swelling of the left testicle of two years' and testicular pain of five days' duration. Physical examination revealed a grapefruit-sized testicular mass, acutely tender on its inferolateral surface. There was no gynecomastia. Orchidectomy was performed. Death occurred ten years seven months later, due to heart disease. No evidence of tumor was found at autopsy.

10. Masson, P.: *Rev. canad. de biol.* **1**:570, 1942.

11. Gynecomastia secondary to a testicular neoplasm may not necessarily be of estrogenic origin; evidence exists that androgens may likewise stimulate mammary growth.

McCullagh, E. P., and Rossmiller, H. R.: *J. Clin. Endocrinol.* **1**:496, 1941.

12. Hunt, V. C., and Budd, J. W.: *J. Urol.* **42**:1242, 1939.

13. Venning, E. H.: *Rev. canad. de biol.* **1**:571, 1942.

14. Masson, P., and Sencert, L.: *Bull. Assoc. franç. p. l'étude du cancer* **12**:555, 1923.

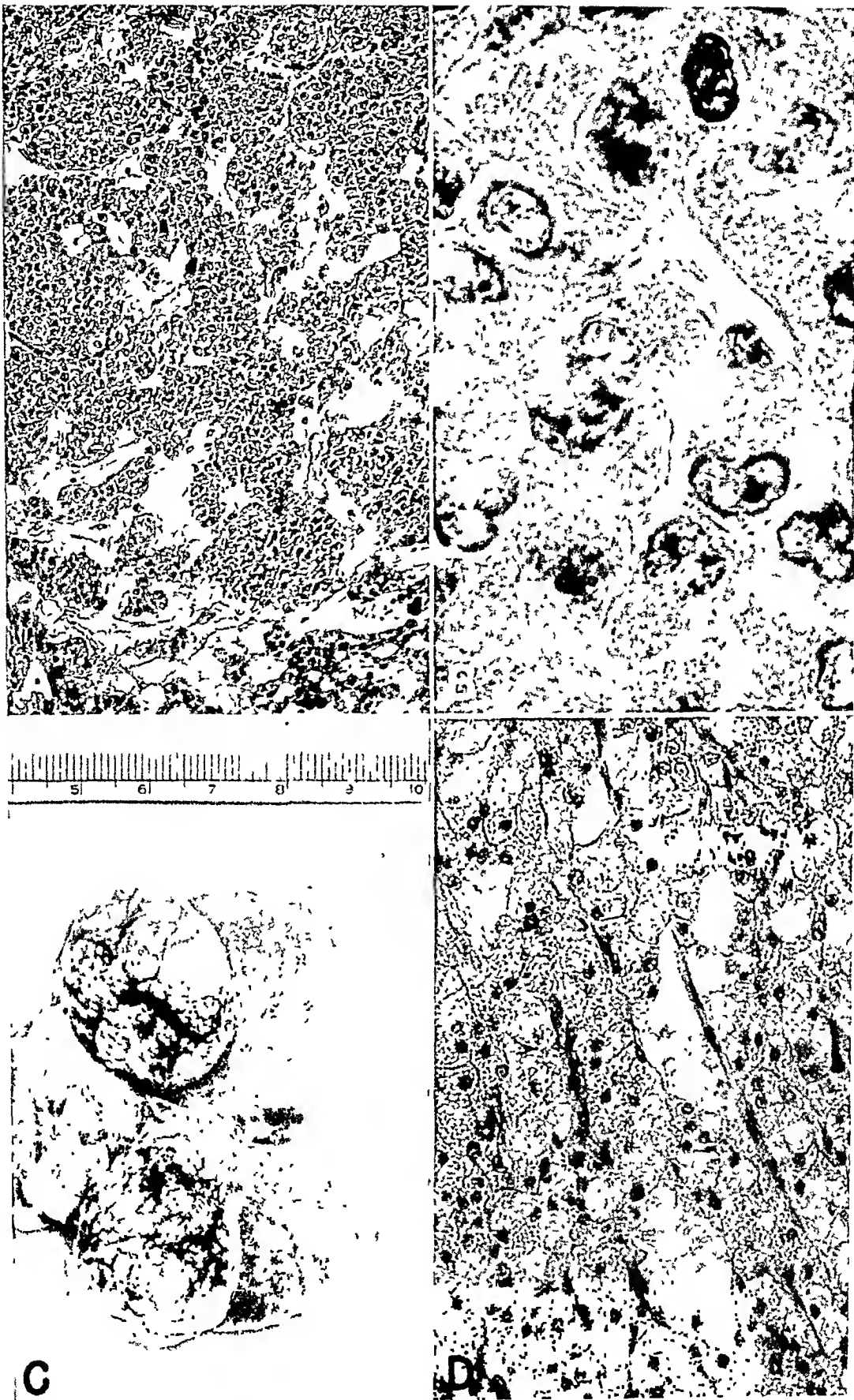


Figure 1

(See legend on opposite page)

The tumor, epididymis and cord weighed 305 Gm. The tumor measured 8 cm. in diameter; it was spherical and encapsulated. On section it was soft and slightly resilient and was composed of translucent grayish tissue lobulated by fibrous trabeculae. Large amounts of clotted blood were present. At the periphery was a rim of opaque yellow tissue. Microscopically, the tumor was composed of cords and sheets of polyhedral cells arranged along blood-containing sinusoid spaces (fig. 1 *A*). The cells were characterized by slightly granular acidophilic cytoplasm so distributed as to form a dense centrum surrounded by a narrow pale halo (fig. 1*B*).¹⁵ The cell borders were distinct. Occasional cells contained small yellow-brown globules in their cytoplasm. The nuclei were oval, bent or wrinkled, and frequently eccentric; they contained a finely beaded reticulum of chromatin and usually one prominent nucleolus. Occasional mitoses were observed.

ADRENAL CORTEX REST TUMORS

Adrenal cortex rests are common in the vicinity of the epididymis. Although Glynn¹⁶ was able to find but one report of aberrant adrenal tissue within the testis proper, Friedman and Ash⁹ stated that such inclusions are frequent.

The identification of adrenal rest neoplasms in the testis is extremely difficult, owing to the striking resemblance of adrenal and interstitial cells. In general, morphologic or endocrinologic features regarded as atypical for the commoner Leydig cell tumor have constituted the criteria for diagnosis. In the following paragraphs are discussed three types of tumor which have been considered to be of adrenal cortex rest origin.

The first type is a rare testicular neoplasm composed largely of cells laden with tiny fat droplets (fig. 1 *D*) and bearing a striking resemblance to adrenal cortex. Although an origin from the latter cannot be excluded, the morphologic aspect is equally consistent with neoplasia of the Leydig cells, for the latter, both in tumors which show typical features elsewhere and in non-neoplastic testicles, may appear as polyhedral lipid-laden cells. Case 2 of the present series (figs. 1 *C* and *D*) illustrates this type. The tumor with the morphologic characteristics of adrenal cortex occurred in a 36 year old man who experienced no clinically manifest endocrine disturbances. It is being classified descriptively as an adrenal cortex-like tumor of the testis; its origin is in doubt.

Since adrenal cortex neoplasms in males can secrete estrogens, one Leydig cell-like tumor of the testis¹⁷ associated with an increase of

15. A fat stain could not be made, owing to the absence of formaldehyde-fixed material.

16. Glynn, E. E.: *Quart. J. Med.* 5:157, 1911.

17. Ostergaard, E.: *J. Clin. Endocrinol.* 7:438, 1947.

Fig. 1.—*A*, interstitial cell tumor (case 1), showing a trabecular pattern. Note wrinkled appearance of nuclei. $\times 200$.

B, interstitial cell tumor (case 1), showing the characteristic dense granular cytoplasmic centrum and the clear peripheral halo. $\times 900$.

C, adrenal cortex-like tumor (case 2). The bisected testicle presents an encapsulated tumor in the superior pole. Dark patches represent areas of hemorrhage.

D, adrenal cortex-like tumor (case 2). $\times 400$.

the estrogen excreted in the urine has been regarded as of adrenal rest origin (second type). Again, however, interstitial cell neoplasia cannot be excluded, since the ability of this cell to produce estrogens is at present undetermined.

Two recently reported bilateral testicular neoplasms¹⁸ present greater claim to authenticity as adrenal rest tumors (third type). These, occurring in males with precocious somatic and genital development, were accompanied by hyperplasia of the adrenal cortex. In each case the neoplastic testicular cells were similar in appearance to the hyperplastic cortical cells. Although in one case^{18a} they contained small amounts of lipid in fine droplets, the tumor cells were characterized by granular cytoplasm and were not of the lipid-laden type.

CASE 2 (adrenal cortex-like tumor of testis).—F. L., a 36 year old white man, complained of pain in the left testicle following trauma two months prior to admission. The pain disappeared temporarily but recurred one month later. Physical examination revealed a firm, tender testis one-third larger than normal. No gynecomastia was present. An Aschheim-Zondek test was "slightly positive" on one occasion and negative on another. Orchidectomy was performed. The patient is alive and well eight years three months after the operation.

The testis, cord and epididymis weighed 40 Gm. The superior pole of the testis contained a round, encapsulated, bright orange-yellow, soft tumor showing focal areas of hemorrhage. It measured 3.3 by 2.7 by 2.0 cm. (fig. 1 C).

Microscopically, the tumor was composed of a diffuse mass of large polyhedral cells supported by a rich capillary network (fig. 1 D). At several points islands of tumor cells were present within the thick capsule composed of hyalinized fibrous tissue. In the larger portion of the tumor the cytoplasm of the cells was laden with tiny fat droplets; in other areas, however, it was densely eosinophilic and free of visible fat. Occasionally a dense centrum of eosinophilic cytoplasm was surrounded by fine peripheral vacuoles. No intracytoplasmic pigment was visible. The tumor nuclei were small, round or oval, and pale, and frequently contained single, fine nucleoli. No mitoses were observed.

TUBULAR "ADENOMA"

The tubular "adenoma" occurs solely in the cryptorchid testicle, where it appears as single or multiple discrete white or yellow nodules. It may have microscopic dimensions or attain the size of a bean.¹⁹

Histologically one encounters closely packed small tubules lying in a scant stroma which may or may not contain Leydig cells (fig. 2). Lining the tubules are cylindric cells with loose granular cytoplasm and crowded oval or elongated nuclei. In a number of cases smaller round cells lie compressed between the taller elements.²⁰ The proliferating

18. (a) Cohen, H.: *Am. J. Path.* **22**:157, 1946. (b) Wilkins, L.; Fleischmann, W., and Howard, J. E.: *Endocrinology* **26**:385, 1940.

19. Ewing, J.: *Neoplastic Diseases: A Treatise on Tumors*, Philadelphia, W. B. Saunders Company, 1940.

20. Chevassu, M.: *Tumeurs du testicule*, Thesis, Paris, no. 193, Paris, G. Steinheil, 1906.

cells may obliterate the tubular lumens, or, on the other hand, may surround small oval or star-shaped openings. Not infrequently the latter contain casts of amphophilic colloid-like material. Transitional stages from adenoma tubules to atrophic tubules lined by typical Sertoli cells are observable in the adjacent parenchyma.

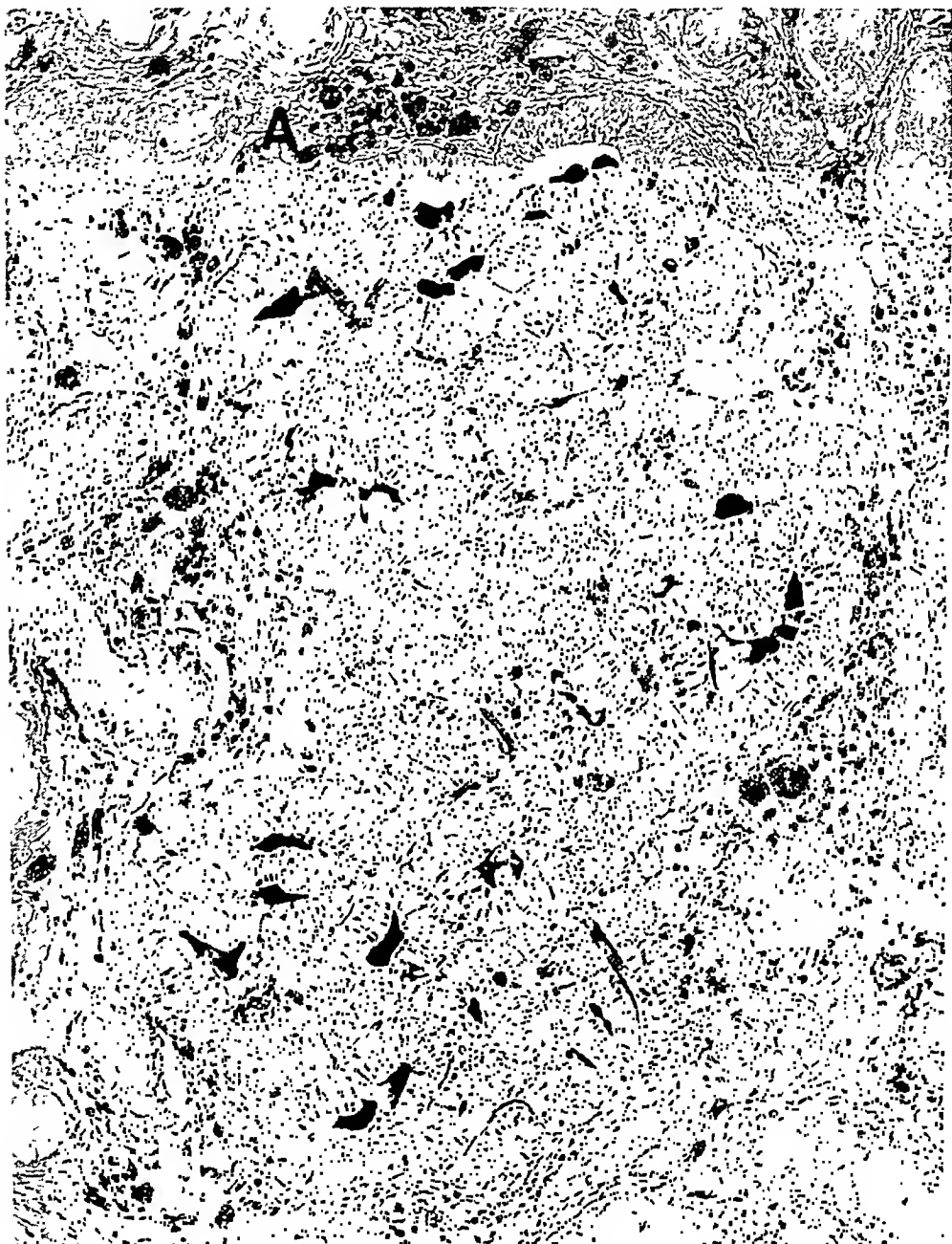


Fig. 2.—Testicular adenoma in a cryptorchid testis. The tubules of the adenoma contain basophilic colloid. Numerous collections of Leydig cells are present (e.g., at *A*). The surrounding testis shows atrophy and fibrosis. Sertoli cells containing large lipid vacuoles line the atrophic tubules. $\times 150$.

The testicular adenoma is of no clinical significance; cancerous transformation has not been reported. Since, in addition, microscopic examination of undescended testicles frequently reveals the presence of adenoma tubules occurring singly as well as in clusters, the designation of these lesions as true tumors appears unjustifiable.²¹

ADULT CARCINOMAS

A small number of testicular carcinomas free of teratomatous elements and composed of cells lacking the staining properties which characterize embryonal cells have been reported. Of these, the tumor of Waterman and Brines,²² composed of faintly basophilic cells arranged in cords with occasional lumen formation, offers no clues as to its origin. The ones described by Bell,²³ which have been accepted elsewhere⁴ as adult carcinomas, we believe may be embryonal, since the author characterizes each as having cells of the germinal type; the published photomicrographs unfortunately are at too low a magnification to enable one to interpret accurately the morphologic features of individual cells.

Adult carcinomas which present evidence of having arisen from the spermatogenic epithelium, the Sertoli cell and the rete epithelium likewise rarely occur.

Spermatocytic Seminoma.—Masson³ has observed 6 round cell carcinomas of the testis which differ from the classic seminoma by the irregularity and smaller size of their cells, their lack of cytoplasmic glycogen and the scantiness of their stroma, which is free of lymphoid infiltration. The striking resemblance of the cells to spermatogenic cells, the marked tendency to invade the seminiferous tubules and the observation in 1 case of the genesis of a neoplastic focus within a tubule are presented as evidence of origin from male germinal epithelium.

Multicystic Adenocarcinoma (? Sertoli Cell Carcinoma).—The adult carcinomas of Stevens and Ewing^{4a} and Stofer^{4b} in addition to solid neoplasia exhibited a papillary cystic pattern closely simulating rete testis. The former tumor grossly spared the rete; in the belief that it arose from adult seminiferous epithelium it was termed adult multicystic adenocarcinoma.

In the present series a similar carcinoma showing diffuse, trabecular and papillary cystic patterns (figs. 3 A, B and D) was encountered in a 60 year old man. Two features of this tumor favored its being inter-

21. Genuinely neoplastic tubular adenomas, which may be histologically indistinguishable from the testicular "adenoma" occur, however, in the ovary; there they are often associated with masculinization.

22. Waterman, J. L., and Brines, O. A.: U. S. Nav. M. Bull. 41:1690, 1943.

23. Bell, P. G.: Brit. J. Surg. 13:282, 1925.

preted as a Sertoli cell carcinoma: 1. In numerous tumor cells in the solid portions were large vacuoles of variable size separated by networks of cytoplasmic septums (fig. 3 C). This appearance recalled that of Sertoli cells in atrophic testicular tubules. Likewise, intracellular fat vacuoles of similar dimensions are prominent in Sertoli cell carcinomas occurring in dogs.²⁴ 2. In the lumens of the cystic spaces and within a number of the lining cells were globules of amphophilic colloid material (fig. 3 D) not unlike that present in the tubular lumens in Sertoli cell adenoma (fig. 2).

If we exclude the so-called testicular adenomas, tumors of Sertoli cell origin have not to our knowledge been described as such occurring in man. They are, however, of not infrequent occurrence in dogs, in which they may be associated with clinical estrogenic manifestations, and in a number of cases an estrogenic substance has been extracted from them.²⁴ Despite this strong evidence that the Sertoli cell may secrete estrogens, histochemical studies done on the testes of mammals other than man and dog have failed to indicate the presence of steroid hormones elsewhere than in the Leydig cells.²⁵ In the present case no light is shed on the problem since there was no clinical evidence that estrogen was secreted by the tumor, nor were assays of hormones made.

CASE 3.—M. F., a 60 year old white man complained of painless swelling of the right testicle of two months' duration. Physical examination revealed a firm, nontender mass in the right scrotum, the size of a double fist. There was no gynecomastia. Orchidectomy was performed. The patient had been symptom-free for approximately three and a half years, when he was admitted to an outside hospital for gastrointestinal complaints and loss of weight. Death occurred from extensive metastatic disease four years eight months after the operation. No biopsy of metastatic lesions or autopsy was performed.

At operation a hydrocele was encountered. The tunica vaginalis was studded with numerous tumor nodules. The testis and epididymis were almost entirely replaced by a hard mass, glistening and yellowish white on the cut surface and twice the size of a normal testicle.

Microscopically the tumor was composed in large part of irregular sheets, cords and strands of epithelial cells separated by abundant collagenous stroma (fig. 3 A and B). The cytoplasm of the neoplastic cells was eosinophilic and nongranular and varied in amount from scant to abundant; often it became condensed to form intracellular hyaline globules, while in some areas droplets and pools of eosinophilic hyaline material lay among the cells. A striking feature was the presence in numerous cells of large round vacuoles²⁶ separated by thin cytoplasmic septums (fig. 3 C). The tumor nuclei varied widely from small round or oval to larger irregular forms; the latter were oval or bent and frequently eccentric. The chromatin was granular and abundant, and numerous mitoses were present. One portion of the

24. Huggins, C., and Moulder, P. V.: *Cancer Research* 5:510, 1945.

25. Pollock, W. F.: *Anat. Rec.* 84:23, 1942.

26. A fat stain could not be made, owing to the absence of formaldehyde-fixed material.



Figure 3

(See legend on opposite page)

tumor consisted of many intercommunicating elongated cystic tubules into which projected single and anastomosing papillary projections of dense stroma (fig. 3 *D*). The cysts were lined by simple flat to columnar or stratified tumor cells. The nuclei of the latter were in general more mature than those of the solid portion, being relatively uniform, oval or elongated, and pale; only occasional mitoses were encountered. In the lumens of the cystic spaces and within a number of the lining cells were large globules of amphophilic colloid-like material (fig. 3 *D*). This resembled closely the eosinophilic material observed in the solid portion of the tumor.

Numerous nests of tumor cells were present within lymphatic channels.

Carcinoma of Rete Testis.—Feek and Hunter²⁷ have reported an adult carcinoma of a 59 year old man showing transitions from rete tubular epithelium to a papillary carcinomatous pattern. In the present series an adenocarcinoma radiating from the hilus into the testicular parenchyma (fig. 4 *A*) was encountered in a man 48 years of age. The manner of growth, from both gross and microscopic points of view, was that of a primary neoplasm; furthermore, the gross localization of the tumor, as well as the microscopic appearance of irregular elongated tubules lined by cells resembling rete epithelium, indicated that an origin from the latter was most probable.

CASE 4.—Y. S., a 48 year old white man, complained of low back pain that radiated down the right leg of five months' duration. The testicles were not abnormal to palpation. Roentgen examination revealed atelectasis of the middle lobe of the right lung. Bronchoscopic examination showed narrowing of the bronchi of the middle and lower lobes of the right lung but no evidence of intrinsic bronchial tumor. An expanding destructive lesion of the ninth rib, which became apparent by roentgenogram seven weeks after admission, was excised. Pathologic examination showed an adenocarcinoma which was simultaneously osteolytic and osteoblastic. Eleven weeks after admission the right testicle became painful, tender and swollen. Physical examination now revealed a hydrocele and a stony-hard testicular mass. An Aschheim-Zondek test was negative. Bilateral orchidectomy was performed. The patient died ten months later with extensive metastatic disease which had been resistant to roentgen therapy. No autopsy was performed.

Pathologic examination of the neoplastic testis revealed a sharply circumscribed, pale brown, scirrhous mass measuring 3 by 1.7 by 2.2 cm. and radiating from the hilus into the parenchyma (fig. 4 *A*). Microscopically, irregular elongated acini separated by abundant loose, poorly cellular collagenous stroma, infiltrated extensively between atrophic testicular tubules (fig. 4 *B*). The tumor cells were cuboidal or peg-shaped and were characterized by abundant eosinophilic cytoplasm

27. Feek, J. D., and Hunter, W. C.: Arch. Path. 40:399, 1945.

Fig. 3.—*A*, multicystic adenocarcinoma of the testis (case 3), showing a diffuse pattern. Abundant collagen is present between groups of tumor cells and between individual tumor cells. Several sclerosed seminiferous tubules remain intact (e.g., at 1). $\times 200$.

B, multicystic adenocarcinoma of the testis (case 3). The tumor nests are isolated by fibrous stroma. $\times 200$.

C, multicystic adenocarcinoma of the testis (case 3). The tumor cells contain various-sized round vacuoles separated by cytoplasmic septums. $\times 800$.

D, multicystic adenocarcinoma of the testis (case 3). A papillary cystic portion is seen. Note the globules of colloid in the small cyst to the left of 1. $\times 100$.

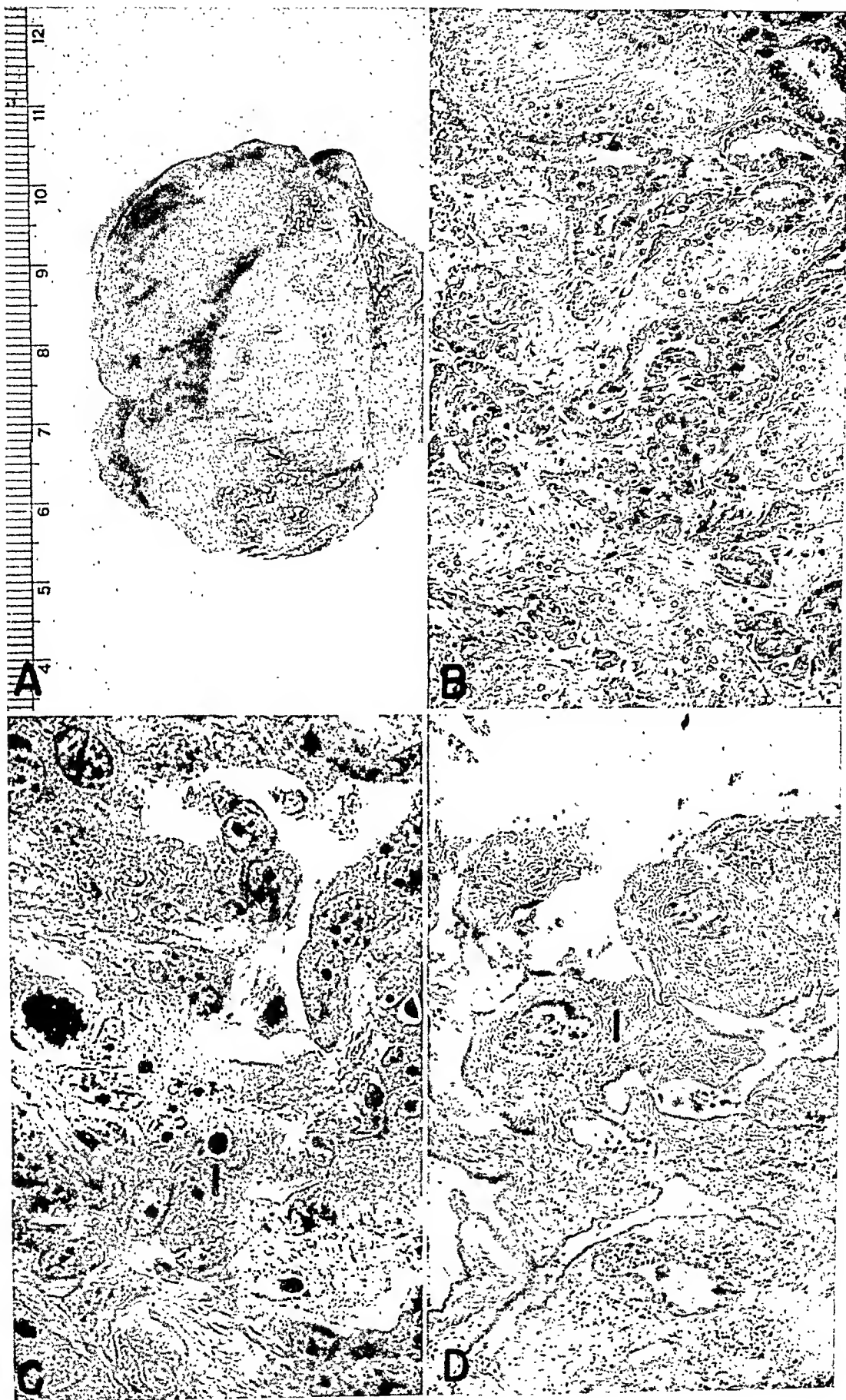


Figure 4
(See legend on opposite page)

and round to oval nuclei containing single or double prominent central nucleoli (fig. 4 C). Large, irregular, hyperchromatic nuclei and mitotic forms were not infrequent. The cytoplasm of numerous cells enclosed various-sized round or irregular, dark brown or eosinophilic globules surrounded by clear halos (fig. 4 C). In the mediastinum testis were numerous tumor acini, lying among and at times penetrating the rete tubules (fig. 4 D). There was no evidence of tumor originating directly from rete epithelium in the sections examined; however, in some areas the rete cells had undergone hypertrophy and strongly resembled the tumor cells.

VASCULAR AND FIBROBLASTIC TUMORS

The rare tumors of the vascular system and of the connective tissue framework of the testis have been reported and collected by Rosenthal²⁸ and Chevassu,²⁹ respectively.

LYMPHOSARCOMA

Lymphosarcoma of the testis occurs rarely in children and adults; it is frequently bilateral. Occasionally, there is associated lymphosarcoma of the skin.²⁹ Although the normal testis is stated to contain no lymphoid tissue,¹⁹ tumors of such tissue which appear to have been primary in the testis have been reported.³⁰

METASTATIC TUMORS

Metastases of tumors are rarely observed in the testicle, a survey of the literature by Helfert and Pinck³¹ disclosing less than 50 instances. Willis³² lists three routes by which neoplasms may spread to the testicle: (1) the veins, with retrograde extension or venous embolism, (2) the lymphatic channels, with retrograde extension, and (3) the arteries, with arterial embolism. The first route has been reported in cases of cancer of the left kidney; the second, in cases of neoplasm involving the retroperitoneal nodes. The most commonly reported artery-borne metastasis is the melanoma.

28. Rosenthal, A. A.: *J. Urol.* **55**:542, 1946.

29. Dockerty, M. B., and Priestly, J. T.: *J. Urol.* **48**:514, 1942.

30. Mathe, C. P.: *J. Urol.* **55**:530, 1946.

31. Helfert, I., and Pinck, B. M.: *J. Urol.* **51**:635, 1944.

32. Willis, R. A.: *The Spread of Tumors in the Human Body*, London, J. & A. Churchill, 1934.

Fig. 4.—A, adenocarcinoma of the rete testis (case 4). A cut surface of the testicle, sectioned in the sagittal plane, shows a discrete tumor mass radiating from the region of the rete.

B, adenocarcinoma of the rete testis (case 4). Irregular elongated tumor acini infiltrating between atrophic seminiferous tubules. $\times 200$.

C, adenocarcinoma of the rete testis (case 4). The tumor cells are characterized by abundant dense cytoplasm and nuclei containing prominent nucleoli. Several dense intracytoplasmic globules surrounded by clear halos are visible (e.g., at 1). $\times 800$.

D, adenocarcinoma of the rete testis (case 4). Tumor acini are seen among the rete tubules (e.g., to the left of 1). $\times 75$.

A review of all cases from 1914 to 1947 in which autopsy was performed on a male dying of a cancerous disease other than an intracranial neoplasm at the Peter Bent Brigham Hospital (over 600 cases) disclosed only 1 case in which a metastatic growth was grossly detectable in a testicle. This was a lymphoma unassociated with a leukemic picture of the peripheral blood. Excluding leukemias, secondary microscopic involvement³³ of the testis was likewise exceedingly rare, occurring only in 2 cases, which were cases of multiple plasma cell myeloma; in each case the testicular infiltration was of the leukemic type.

SUMMARY

The interstitial cell tumor is discussed from clinical and pathologic points of view. One case is reported.

A large number of miscellaneous testicular neoplasms were collected in a fairly extensive, although incomplete, review of the literature; they are classified and discussed briefly.

Three rare tumors, an adrenal cortex-like tumor of the testicle, a multicystic adenocarcinoma and an adenocarcinoma of the rete testis, are reported.

33. Microscopic examination of one or both testicles was done in approximately 45 per cent of the total number of cases.

SOME PROBLEMS RELATED TO THE ORIGIN AND MEANING OF PITUITARY GLAND TUMORS

I. COSTERO, M.D.

Professor at the Universidad Nacional; Pathologist at the Instituto Nacional de Cardiología
MEXICO CITY

IN THE course of several years, Dr. Clovis Vincent, neurosurgeon at the Pitié Hospital in Paris, and Drs. Clemente Robles and Mariano Vázquez, at the General Hospital, Mexico City, have been providing me with abundant material in addition to the routine specimens received in the department of pathology of the National Institute of Cardiology; this has enabled me to examine more than 3,000 intracranial tumors. Among these belong the 150 specimens of pituitary gland tumors which are the basis for the data presented in this paper. These data refer to the following matters: (1) the interpretation of the polymorphism of pituitary gland tumors; (2) the exhaustion of the acidophilic adenoma (oligochromic adenoma); (3) the polymorphism of the chromophobe adenoma (adenoeptihelioma); (4) the solitary suprasellar cyst (archeoblastoma); (5) the limitation of the concepts of adamantinoma and teratoma.

INTERPRETATION OF THE POLYMORPHISM OF PITUITARY GLAND TUMORS

Pituitary gland tumors commonly offer difficulties for differential diagnosis because of their extreme cellular variety. In order to interpret correctly the character of the tumor cells in each case, and in order to recognize their reciprocal relations, it is necessary to review the genetic potentialities of the embryonal anlagen of the hypophysis.

Rathke's pouch begins its development when the human embryo achieves the length of 3 mm. (Waterston¹) and is found completely formed toward the fourth week of intrauterine life; the infundibulum appears in the 8 mm. embryo, and its juxtaposition with Rathke's pouch is completed in the 21 mm. embryo; a little later, when the embryo reaches the length of 32 mm., Rathke's pouch becomes definitely isolated from the oral cavity and forms the pituitary vesicle, which is lined by

From the Department of Pathology of the National Institute of Cardiology, and the University of Mexico School of Medicine.

1. Waterston, D.: Tr. Roy. Soc. Edinburgh 55:125, 1926.

simple columnar epithelium; behind it lies the infundibulum, and the whole structure is correctly limited by a membrane formed by the meningeal covers.

The prostomic epithelium, which has been displaced so early into the skull base in order to form the pars buccalis of the hypophysis, conserves its multiple morphologic potentialities for a long time. These potentialities may develop in three manners: typically, atypically and abnormally (table 1).

I consider as typical potentialities those which determine the development of the pars buccalis sive glandularis of the hypophysis, involving (a) the formation of chromophobe, acidophilic and basophilic cells, which are normally found in the pars distalis, and (b) the conservation

TABLE 1.—*Potentialities of the Pituitary Vesicle*

Typical	(a) Formation of chromophobe, acidophilic and basophilic cells	Pars distalis
	(b) Conservation of cavities lined by either columnar, cuboidal or squamous epithelium which may or may not contain a colloid	Pars juxtaneuralis
Atypical	(c) Formation of pseudostratified or stratified columnar epithelium, with or without cilia, with or without tendency to secrete mucin	Hamartia
	(d) Formation of stratified squamous epithelium, with or without tendency to cornification	
	(e) Invagination of the epithelium with formation of simple or branched tubular glands, as well as acinotubular glands with or without tendency to secrete mucin	
Abnormal	(f) The epithelium of the pituitary vesicle conserves its simple columnar arrangement without forming cavities	Chorista
	(g) It induces formation of lymphoid follicles of tonsillar type in the adjacent connective tissue	
	(h) Likewise, it induces the formation of cartilage	
	(i) It transforms itself into enamel germs which may secrete a calcifiable albuminous substance	

of cavities lined by either columnar, cuboidal or squamous epithelium, which may or may not contain a colloid, as seen in the pars juxtaneuralis.

As atypical potentialities are considered (c) formation of pseudostratified or stratified columnar epithelium with or without cilia and with or without secreted mucin, (d) formation of stratified squamous epithelium with or without tendency to cornification and (e) invagination of the epithelium forming simple or branched tubular glands as well as acinotubular glands with or without tendency to secrete mucin. These three potentialities may become manifest in normal glands and represent a mistake of development, i. e., hamartia, according to Albrecht's nomenclature²; such structures are generally found in the pars juxtaneuralis and have a tendency to disappear gradually, so that they are common in children and rare in adults.

2. Albrecht, E.; Frankfurt. Ztschr. f. Path. 1:221, 1907.

I consider as abnormal potentialities those which represent displacements of embryonal anlagen, i. e., chorista, according to Albrecht, examples of which occur when (f) the epithelium of the pituitary vesicle conserves its simple columnar arrangement without forming cavities, (g) it induces formation of lymphoid follicles of tonsillar type in the adjacent connective tissue, (h) it likewise induces formation of cartilage and (i) it transforms itself into enamel germs which may secrete a calcifiable albuminous substance.

All the potentialities described in the foregoing paragraphs may appear in the tumors. The typical potentialities give rise to adenomas, while the atypical and abnormal ones manifest themselves chiefly in the tumors derived from the lining epithelium.

EXHAUSTION OF THE ACIDOPHILIC ADENOMA (OLIGOCHROMIC ADENOMA)

The existence of pituitary adenomas formed by cells similar to the eosinophilic cells of the normal hypophysis but containing small amounts of specific acidophilic substance has been pointed out by many authors. Poindecker³ described the so-called pregnancy cell adenoma. Dott and Bailey⁴ and Bailey and Davidoff⁵ found that in many acidophilic adenomas the granulations were so weakly stained that their demonstration was difficult; in other tumors they observed that the number of granulated cells was small and that occasionally the specific granulations were absent as in the case of Poindecker's tumor. Kraus⁶ called those tumors which contain few cells with typical acidophilic granulations transitional adenoma. Cushing⁷ found a mixed variety of adenoma, formed in some parts by chromophobe elements and in others by cells with acidophilic granulations. All these forms have been placed together by Costero and Berdet⁸ under the name of oligochromic adenoma; at the same time, they added the description of three histologic groups: exhausted acidophilic adenoma, grouped cell adenoma and megalocytic adenoma.

3. Poindecker, H.: *Wien. klin. Wchnschr.* **26**:745, 1931.

4. Dott, N. M., and Bailey, P.: *Brit. J. Surg.* **13**:314, 1925.

5. Bailey, P., and Davidoff, L.: *Am. J. Path.* **1**:185, 1925.

6. Kraus, E. J., in Henke, F., and Lubarsch, O.: *Handbuch der speziellen pathologischen Anatomie und Histologie*, Berlin, Julius Springer, 1926, vol. 8. p. 810.

7. Cushing, H.: *Intracranial Tumors*, Springfield, Ill., Charles C Thomas, Publisher, 1932.

8. Costero, I., and Berdet, H.: (a) *An. Esc. nac. de cien. biol.* **1**:67, 1938; (b) *Estudio anatómico de 135 tumores de la hipófisis y dell tracto hipofisario*, Monograph of the Sociedad Médica del Hospital General, México, 1939; (c) *Gac. méd. de México* **69**:286, 1939.

The study of a sufficient number of specimens suggests that, of all the morphologic types classified under the name of oligochromic adenoma, only these last three represent the terminal stages of three different types of evolution of acidophilic adenoma. Following this criterion, I consider them to be the only static or true oligochromic adenomas. Their characteristic features are described in the following paragraphs.

Apparently, the most common phenomenon is the gradual loss of the cellular capacity to elaborate specific granulations; in this way, the formation of the exhausted acidophilic adenoma is reached through several intermediate forms. To these forms belong some of those described by Dott and Bailey, the transitional adenoma of Kraus, and Poindecker's tumor.

Figure 1 reproduces, with low magnification, the aspect of a typical acidophilic adenoma in which the granulations have been stained by the method of Bensley. All the cells of the tumor appear with their cytoplasm obscured by the acidophilic substance. Note the density of the neoplastic parenchyma, the tendency of the individual cell to become polyhedral through pressure and the central position of the nucleus.

Figure 2 shows a transitional adenoma in which the cells have abundant granulations with little affinity for the acidophilic substance, as becomes apparent when their color is compared with the intensity of the staining of the red blood cells. Observe that the tumoral elements have the same dense disposition, polyhedral shape and central nucleus as those of the typical acidophilic adenoma.

Another variety of transitional adenoma is shown in figure 3; in this one, only a small number of cells contain specific granulations, and high magnification is necessary in order to demonstrate them in the photomicrograph. The position of the nucleus and the arrangement of the cells is the same as in the typical acidophilic adenoma.

The reason for emphasizing the arrangement and the shape of the cells in each instance is that, in my opinion, they permit one to ascertain with a sufficient degree of correctness the hormonal activity of the adenoma without resorting to specific staining for granules. It is sufficient to remember the following general rule: The progressive exhaustion of the capacity of the neoplastic cells for elaborating specific acidophilic granulations is announced by these three morphologic phenomena—looseness of the neoplastic parenchyma, formation of spheroidal cells with homogeneously acidophilic cytoplasm and displacement of the nucleus toward the periphery of the cytoplasm.

A good example of such morphologic changes is found in figure 4, reproducing an oligochromic adenoma of the variety described by Poindecker. The tumor is divided into lobules by the huge vessels in

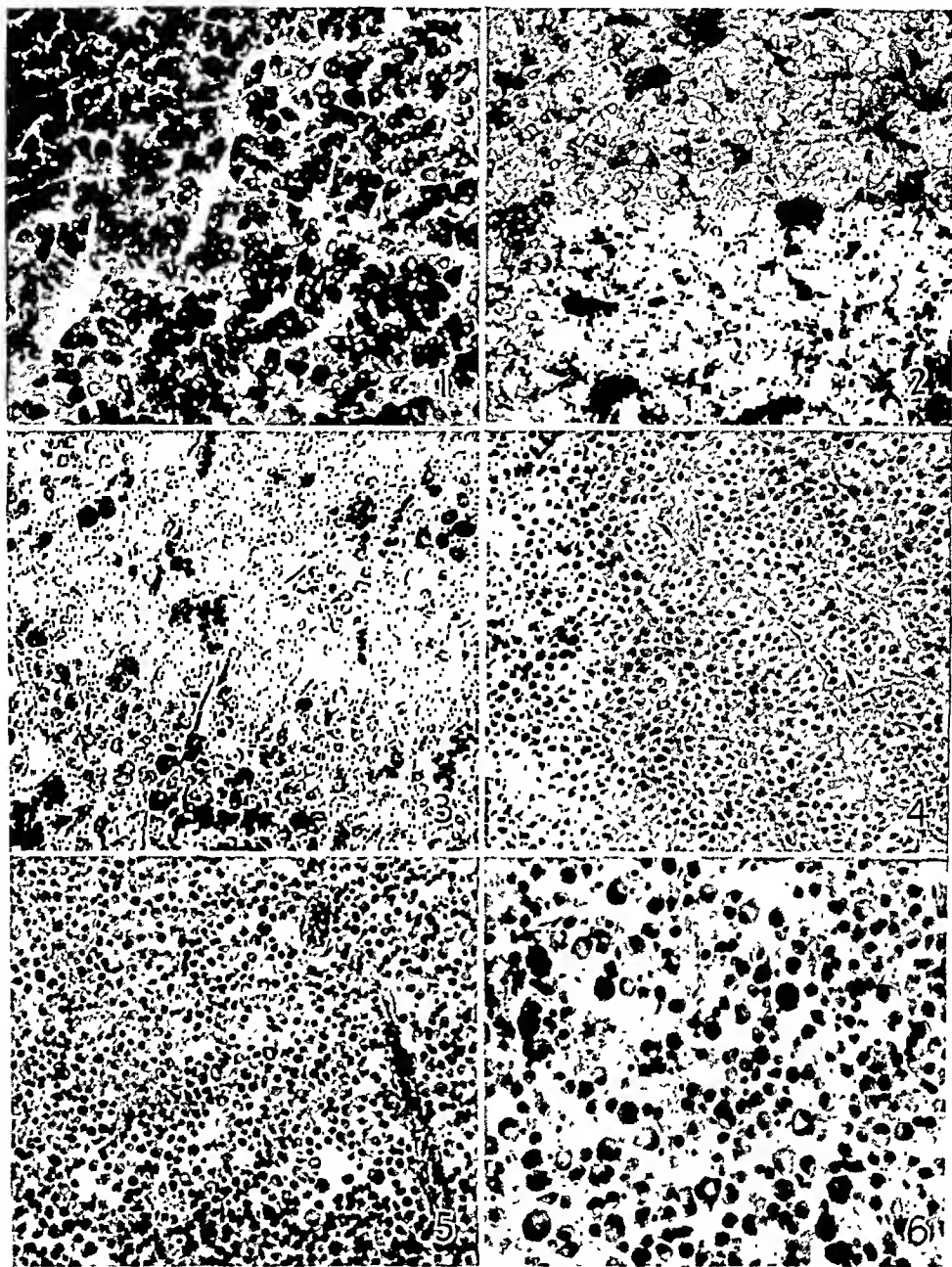


Fig. 1.—Typical acidophilic adenoma; Bensley's crystal violet and orange G stain; $\times 57$.

Fig. 2.—Transitional adenoma in which the granulations have little affinity for the acidophilic substance; Bensley's crystal violet and orange G stain; $\times 57$.

Fig. 3.—Transitional adenoma in which the capacity of the cells to produce acidophilic granulations is greatly reduced; Bensley's crystal violet and orange G stain; $\times 99$.

Fig. 4.—Oligochromic adenoma of the variety described by Poindecker; hematoxylin and eosin; $\times 71$.

Fig. 5.—Exhausted acidophilic adenoma; hematoxylin and eosin; $\times 28$.

Fig. 6.—Exhausted acidophilic adenoma under higher magnification; hematoxylin and eosin; $\times 85$.

the stroma. The cells lying close to the vessels have the form of a prism, and their cytoplasm shows intense acidophilia; the central portion of the lobules has loose arrangement and is formed by spheroidal cells in which the nucleus tends to take an eccentric position. The specific strains were unable to demonstrate any granulations in this tumor.

In the exhausted acidophilic adenoma all the cells have a rounded shape, an eccentric and frequently lobulated nucleus and limits sharply outlined, owing to the intense eosinophilia of the cytoplasm; they lie within an albuminous mass, in which they seem to float and which contains a delicate network of blood vessels. The general aspect of the slides when observed under low power (fig. 5) is more suggestive of a smear of bone marrow than of a section of a solid tissue. At higher magnification the histologic features described become apparent (fig. 6).

Megalocytic adenoma is rare, and this may be the reason why its histogenesis is difficult to understand. The tumor cells are much bigger than in any other variety of pituitary adenoma (fig. 7). The cytoplasm is intensely eosinophilic and homogeneously grumous; the nucleus, which is rarely lobulated, is constantly found in a peripheral position. The high density of the cellular arrangement makes the cells adopt a polyhedral shape with rounded edges. Under low power a slide of megalocytic adenoma has a certain similitude to a transverse section of striated muscle.

The arrangement of the large cells is due to the disposition of the blood vessels; thus it appears uniform and regular when the capillaries form a homogeneous network as in figure 7; when the vessels are parallel, the cells form long cords which anastomose like liver trabeculae (fig. 8).

I have not been able to find transitional forms between the typical acidophilic adenoma and the megalocytic adenoma. Probably the latter is formed by acidophilic elements which suddenly have lost their capacity for elaborating specific granulations; i. e., I suppose, the megalocytic adenoma is a frustrated form of acidophilic adenoma.

The grouped cell adenoma seems to originate by an exaggeration of the capacity of the acidophilic cells for elaborating interstitial albuminous substance. This capacity is already manifest in the exhausted adenoma and is comparable to that existing in the pars juxtaneuralis of the normal hypophysis. In the grouped cell adenoma (fig. 9) the stroma appears to be formed by a weakly and uniformly acidophilic colloid substance regularly interspersed between the tumor cells, which retracts a little with the substances used in histologic technics. The cells are arranged in small "isogenic groups" in a characteristic manner.

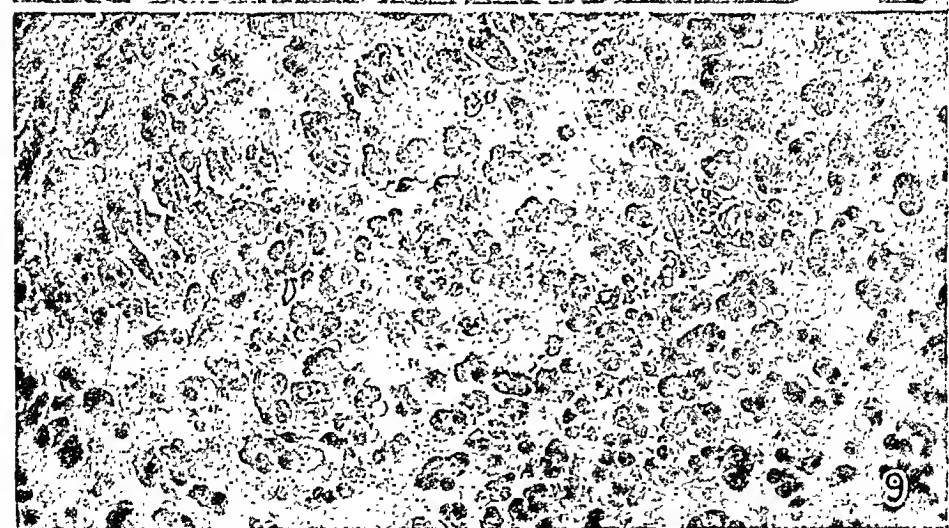
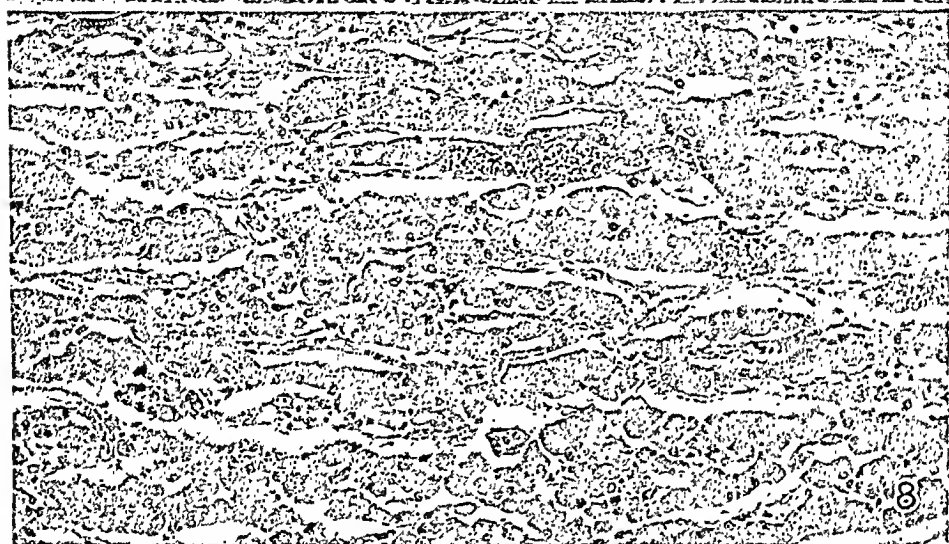
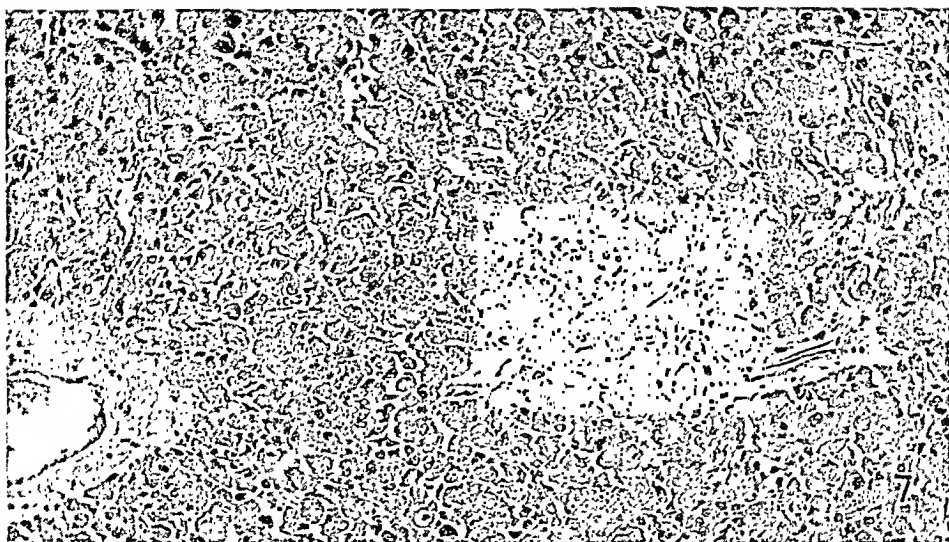


Fig. 7.—Megalocytic adenoma in which the capillaries form a homogeneous network; hematoxylin and eosin; $\times 50$.

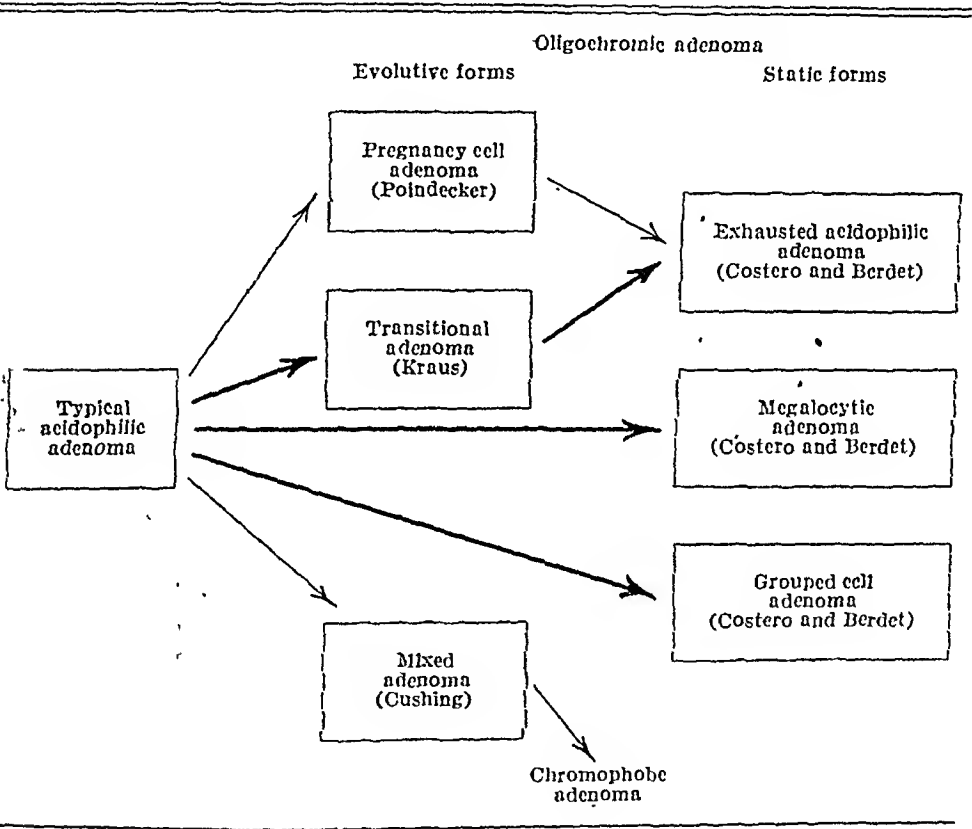
Fig. 8.—Megalocytic adenoma in which the capillaries are parallel; hematoxylin and eosin; $\times 50$.

Fig. 9.—Grouped cell adenoma; hematoxylin and eosin; $\times 100$.

Table 2 shows graphically the probable relationship between the different morphologic varieties of oligochromic adenoma.

Finally, I should like to touch briefly the theme of clinicopathologic correlations. The exhausted acidophilic adenoma is regularly found in a patient with frustrated acromegaly; it does not present any sign of intrinsic malignancy. The most common feature of megalocytic adenoma is the replacing of an acromegaloid syndrome by the syndrome of an intracranial tumor with slow but progressive growth, causing ocular

TABLE 2.—*Probable Relations Among the Outstanding Morphologic Varieties of the Oligochromic Adenoma*



disturbances, diencephalic symptoms and signs of intracranial hypertension. The clinical behavior of grouped cell adenoma is like that of chromophobe adenoma.

POLYMORPHISM OF THE CHROMOPHOBE ADENOMA
(ADENOEPITHELIOMA)

The chromophobe adenoma must be considered, in spite of its wide polymorphism, as one sole variety of tumor. The constituent cells show an extensive variety of shapes; the starting point of these variations seems to be in the columnar cells, which mimic the lining cells of the pituitary vesicle. Passing sometimes through an intermediate

stage which resembles the chromophobe cells, they end by forming dense masses closely similar to cords of stratified squamous epithelium. This is why Río Hortega^{9a} has classified these tumors as adenoepithelioma.

The following graphic exposition of the main morphologic varieties of adenoepithelioma has been arranged in accord with the degree of evolution of the neoplastic cells.

The section reproduced in figure 10 belongs to an adenoepithelioma of columnar cells similar to those of the pituitary vesicle but arranged in irregular series instead of cavities; the tumor is so fragile that the entire parenchyma appears infiltrated with hemorrhages.

In the adenoepithelioma shown in figure 11, the cells are also columnar in shape, but they are disposed in very dense cords; the fetal character of these cells has already been recognized by Kraus.⁶

It may happen that the columnar cells of the tumor become arranged similarly to glandular ducts, as illustrated in figure 12, which represents an evolution more advanced toward nonspecific differentiation; i. e., the structures reproduced in this tumor are not at all similar to those of the functional portion of the normal hypophysis.

Compare that picture with the one shown in figure 13, another adenoepithelioma of glandular aspect, in which many of the tubular formations have already become transformed into solid trabeculae. The columnar form of the cells has given place to a cuboidal or polyhedral one, and many parts of the tumor resemble the pars distalis of the normal hypophysis. This picture would correspond to a transitional stage between the columnar cell adenoepithelioma and the adenoepithelioma formed by chromophobe cells.

The genetic relationship between the embryonic columnar cells and the chromophobe cells of pituitary tumors must be so close that even in the typical chromophobe adenoma, as the one of figure 14, perfectly developed series of columnar cells may be found; the pseudostratified epithelium with cilia shown in figure 15 was found across the tumoral parenchyma; possibly it may have been lining a cavity which was invaded by the adenoma and caused to disintegrate.

The differentiation of the columnar epithelium may be as pronounced as in the case of a cyst included in another chromophobe adenoma (fig. 16), in which there were not only cilia but also mucinous cells among the epithelium; a connective membrane has developed, which contains tubular glands. All these structures together form a picture similar to the mucosa of the higher respiratory pathways. One therefore deals with an association of chromophobe adenoma and choristoblastic formations of teratoid type.

9. del Río Hortega, A. P.: (a) *An. Casa de Salud Valdecilla* 5:3, 1934; (b) *Monograph, Arch. argent. de neurol.*, 1941.

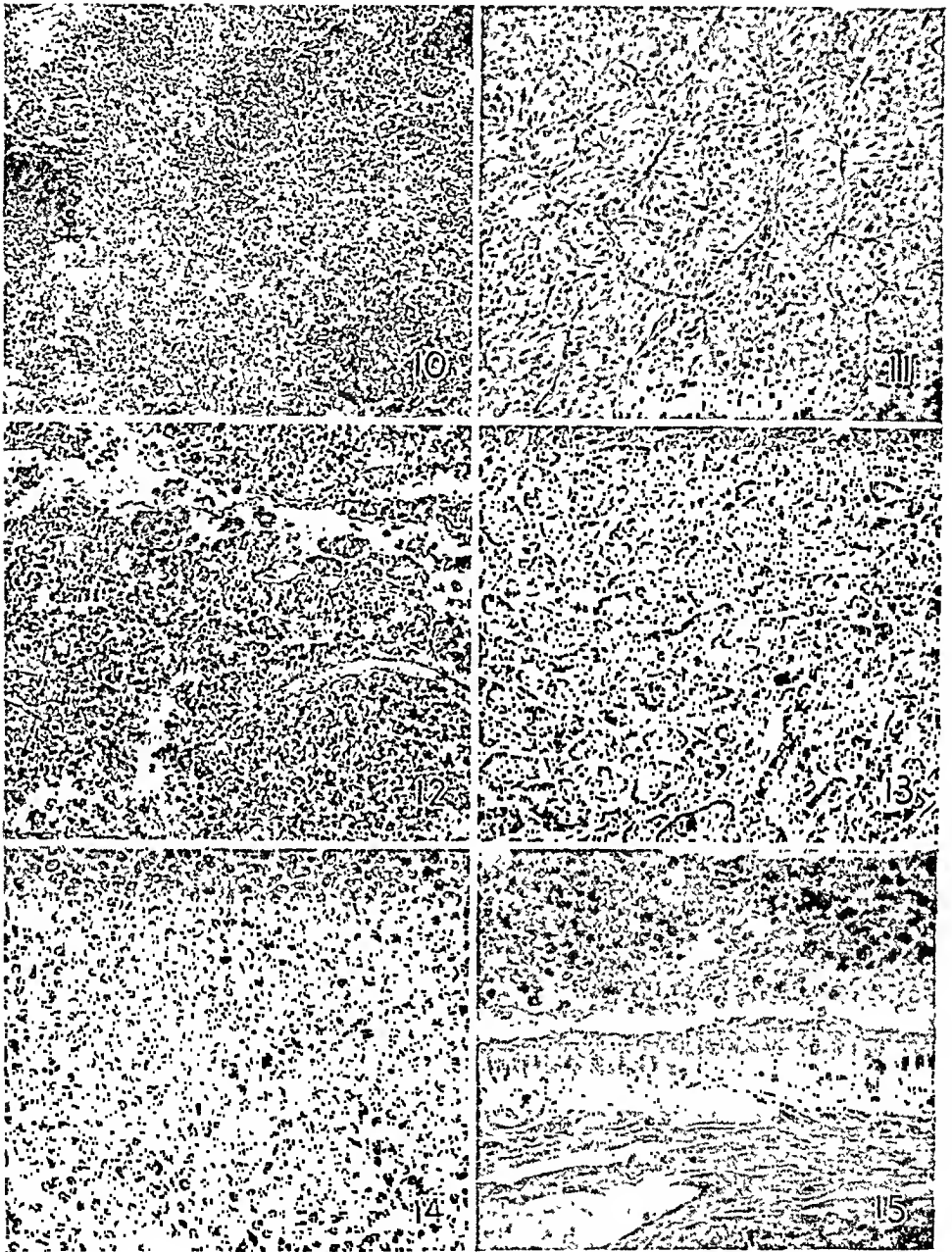


Fig. 10.—Adenoepithelioma with columnar cells; hematoxylin and eosin; $\times 28$.

Fig. 11.—Adenoepithelioma with columnar cells arranged to form dense cords; hematoxylin and eosin; $\times 71$.

Fig. 12.—Adenoepithelioma with columnar cells arranged in such a manner as to resemble glandular ducts; hematoxylin and eosin; $\times 28$.

Fig. 13.—Another adenoepithelioma of glandular aspect; hematoxylin and eosin; $\times 28$.

Fig. 14.—Typical chromophobe adenoma; hematoxylin and eosin; $\times 99$.

Fig. 15.—Pseudostratified epithelium with cilia that may have lined a cavity which was invaded by adenoma and caused to disintegrate; hematoxylin and eosin; $\times 99$.

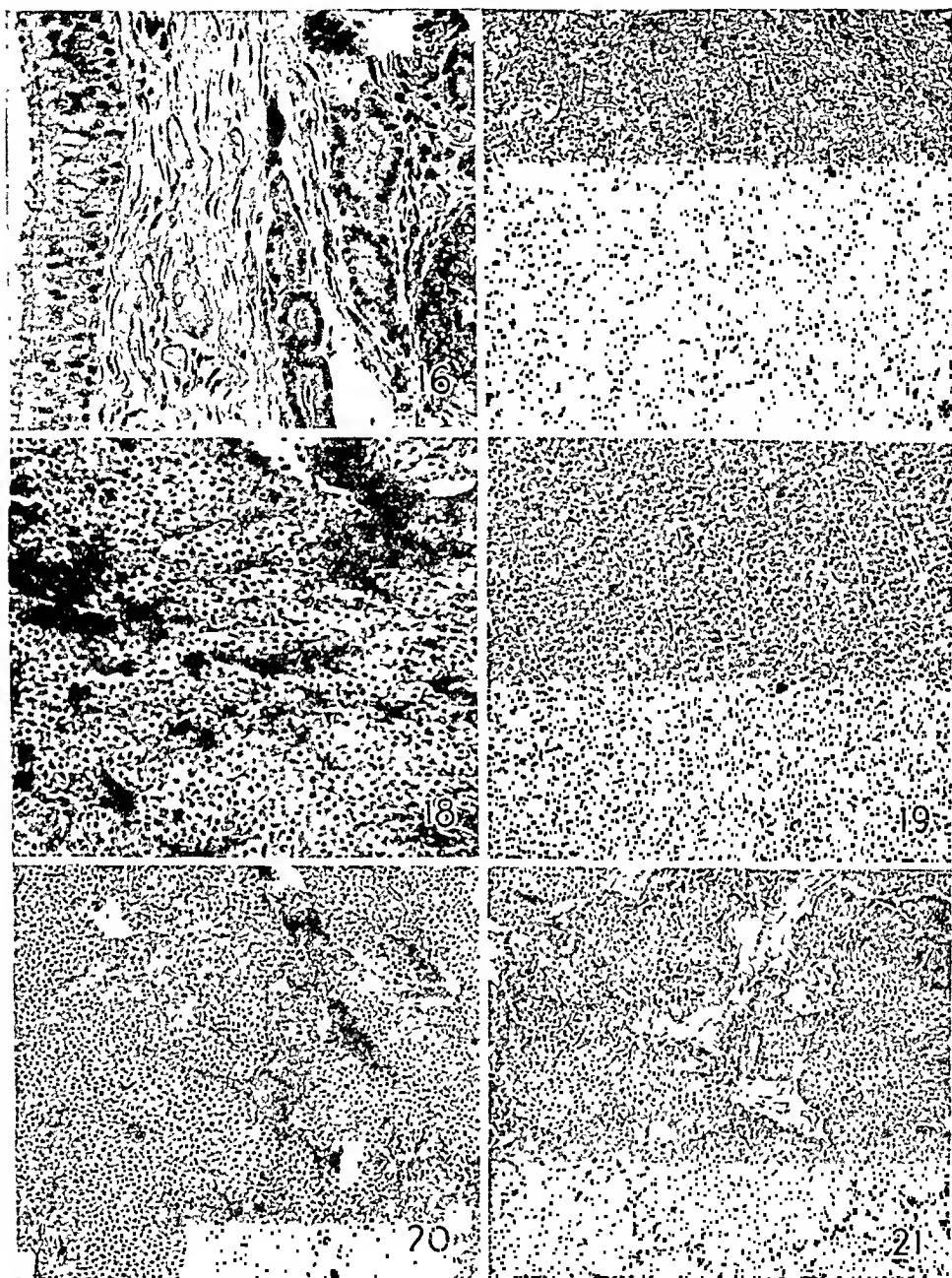


Fig. 16.—Chromophobe adenoma associated with choristoblastic formations of teratoid type; hematoxylin and eosin; $\times 85$.

Fig. 17.—Alveolar adenoma, a variety of chromophobe adenoma; hematoxylin and eosin; $\times 28$.

Fig. 18.—Clear cell adenoma, a variety of chromophobe adenoma; hematoxylin and eosin; $\times 57$.

Fig. 19.—Chromophobe adenoma forming dense epithelial cell cords; hematoxylin and eosin; $\times 28$.

Fig. 20.—Chromophobe adenoma forming anastomosing lobules; hematoxylin and eosin; $\times 28$.

Fig. 21.—Chromophobe adenoma that appears as though formed of independent lobules of squamous epithelium; hematoxylin and eosin; $\times 28$.

The alveolar adenoma, described by Dott and Bailey,⁴ is easily recognized by the smallness and the lymphoid aspect of its cells (fig. 17). The clear cell adenoma of Roussy and Oberling¹⁰ (fig. 18) is characterized by the transparency of the cytoplasm. Both tumors are varieties of the chromophobe adenoma and probably represent degenerative phenomena.

The tendency of the chromophobe adenoma to form dense epithelial structures, without glandular character, is illustrated in the three following figures. In figure 19 the neoplastic parenchyma is homogeneous but divided by scarce stroma into dense cell cords of considerable size. In figure 20 these cell cords are well defined and form anastomosing lobules, even though the cells still conserve some characters resembling chromophobe cells. In figure 21 the tumor appears to be formed by independent lobules of squamous epithelium, although there are no intercellular bridges or cornification.

TABLE 3.—*Classification of the Different Morphologic Varieties of Adenoepithelioma According to the Degree of Evolution of Their Cells*

Adeno- epithelioma (chromophobe adenoma)	With columnar cells	<div><div>Loose</div><div>Dense fetal cell adenoma, Kraus</div><div>Tubular</div></div>
	With chromophobe cells	<div><div>Tubular</div><div>With inclusions of columnar epithellum</div><div>Degenerative forms (alveolar adenoma, Dott and Bailey; clear cell adenoma, Roussy and Oberling)</div></div>
	With polyhedral cells	<div><div>Cells grouped in cords</div><div>Cells grouped in anastomosing lobules</div><div>Cells grouped in independent lobules</div></div>

Malignant chromophobe adenoma which is capable of destructive growth and of metastasizing into the lymph glands is rare; there are only 2 cases in our series. The cancerous character is apparent (more than in the cellular variety) in the frequency of mitotic divisions and in the ample zones of necrosis developed in the tumor.

The data exposed in this chapter are summarized in table 3.

SOLITARY SUPRASellar CYST (ARCHEOBLASTOMA)

The pituitary vesicle of the embryo, detached from the primitive oral cavity, may persist either totally or partially in the adult person, thus constituting an intracranial cyst situated in the region of the sella turcica. Tumors of this type have been studied by many authors, among them Langer,¹¹ Tannenheim,¹² Learmonth and Kernohan,¹³

10. Roussy, G., and Oberling, C.: Presse méd. **92**:1799, 1933.
11. Langer, F.: Ztschr. f. Heilk. **13**:57, 1892.
12. Tannenheim, O.: Wien. klin. Wchnschr. 1897.
13. Learmonth, J. R., and Kernohan, J. W.: S. Clin. North America **11**:835, 1913.

Divry and Christophe,¹⁴ Vampre,¹⁵ Bailey¹⁶ and Río Hortege.⁹ The solitary suprasellar cyst is variable in size; it contains a fluid in which float abundant cholesterol crystals, often of a greenish color owing to the presence of hematic pigment; the cavity is always lined by cornified squamous epithelium.

The cornification of the lining of the cyst does not happen according to the ordinary manner but follows a peculiar morphologic process, which has been studied by Bailey¹⁷; Costero and Berdet^{8b} have added a few data to this description. A portion of cornified cells is detached and falls into the fluid of the cyst; the vast majority of them, however, are displaced into the connective tissue which surrounds the epithelium; there they provoke the appearance of foreign body giant cells. Later, the cornified masses become calcified, sometimes without losing their histologic arrangement; the giant cells canalize the calcified masses; through the newly formed channels vascular buds penetrate, and finally the cornified layer, embedded in the connective tissue, is completely substituted by bony trabeculae. The following pictures illustrate the different phases of this remarkable process.

In figure 22 one sees a part of the squamous epithelium which lines the wall of a suprasellar cyst. The greater part of the cornified cells form clumps situated toward the cavity; but some cells, marked by an arrow, are clumped together in the basal part of the epithelium next to the connective tissue membrane; this membrane shows a dense lymphoid cell infiltration.

Figure 23 shows at higher magnification the aspect of the masses of cornified cells which are displaced into the connective tissue and surrounded by foreign body giant cells. Note the characteristic stratified disposition of the stratum corneum and the partial conservation of its cellular structures.

A more advanced stage is reproduced in figure 24; the cornified masses, still showing remains of the structures mentioned before, appear in an advanced stage of calcification; close to them there is incompletely formed bony tissue.

Figure 25 derives its interest from the fact that it shows in miscellaneous disposition cornified masses with their characteristic structure, others in different periods of calcification, and bony trabeculae completely formed.

14. Divry, P., and Christophe, L.: *J. de neurol. et de psychiat.* **30**:520, 1930.

15. Vampre, E.: *Bol. Soc. de med. e cir. São Paulo* **14**:149, 1930.

16. Bailey, P., in Penfield, W.: *Cytology and Cellular Pathology of the Nervous System*, New York, Paul B. Hoeber, Inc., 1932, vol. 3, p. 1131; *Intracranial Tumors*, Springfield, Ill., Charles C Thomas, Publisher, 1933.

17. Bailey, P.: *Ann. Surg.* **74**:501, 1921.

The solitary suprasellar cyst is always an intrinsically benign formation, but its lining epithelium may show signs of proliferation, especially in the form of budding with dense periphery and loose center, in a manner similar to that of the adamantinoma. Even when the cyst is of great size, it may be well tolerated and the patient may not show important signs of intracranial hypertension. This is explained by the fact that the cyst begins its formation in an early period of the embryonal development, growing simultaneously with the brain and the other parts of the skull, so that the phenomena produced are those of adjustment and not those of compression. As the pituitary vesicle becomes a nonfunctional cyst, the complete or almost complete lack of pars glandularis is frequently manifested in the form of Fröhlich's syndrome. Basing their decision on this sequence of events and on the clinical frequency of these tumors, Costero and Berdet^{8b} have separated the solitary suprasellar cyst from the craniopharyngioma, giving to it the name "archeoblastoma."

LIMITATION OF THE CONCEPTS OF ADAMANTINOMA AND TERATOMA

The atypical potentialities of the columnar epithelium lining the wall of the embryonal pituitary vesicle are already manifest in the normal hypophysis. More than a third of normal persons have squamous epithelium in the hypophysis (Erdheim¹⁸; Simonds¹⁹; Kiyono²⁰; Pérez Lista²¹; Susman²²). It is possible to find columnar epithelium with goblet cells (Peremeschko²³; Bryant²⁴; Guizzetti²⁵; Kiyono²⁰; Rasmussen^{26a, b}) or tubular glands (Erdheim¹⁸; Bevacqua²⁷; Guizzetti²⁵; Lewis and Lee²⁸; Rasmussen^{26c}; Thom²⁹). This means that one must be careful in interpreting the complex tumors derived from the epithelium of the pituitary vesicle, since it is so highly multipotent.

For example, the squamous epithelium of some normal glands and that appearing in archeoblastoma, epithelioma and carcinoma of the pituitary area often show a loose central portion and a dense periphery, as do the enamel germs. I feel that this feature alone is not sufficient

18. Erdheim, J.: *Ztschr. f. Path.* **4**:215, 1910.

19. Simonds, J. P.: *Endocrinology* **50**:766, 1922.

20. Kiyono, H.: *Virchows Arch. f. path. Anat.* **259**:252, 1926.

21. Pérez Lista, M.: *Bol. Soc. españ. de biol.* 1931.

22. Susman, W.: *Brit. J. Surg.* **19**:571, 1932.

23. Peremeschko, W.: *Virchows Arch. f. path. Anat.* **38**:329, 1867.

24. Bryant, W. S.: *Anat. Rec.* **11**:25, 1916.

25. Guizzetti, P.: *Sperimentale, Arch. di biol.* **79**:73, 1925.

26. Rasmussen, A. T.: (a) *Endocrinology* **12**:129, 1928; (b) *Anat. Rec.* **41**:273, 1929; (c) **55**:139, 1933.

27. Bevacqua, A.: *Anat. Anz.* **38**:3, 1911.

28. Lewis, D., and Lee, F. C.: *Bull. Johns Hopkins Hosp.* **41**:241, 1927.

29. Thom, W.: *Arch. f. mikr. Anat.* **57**:632, 1931.

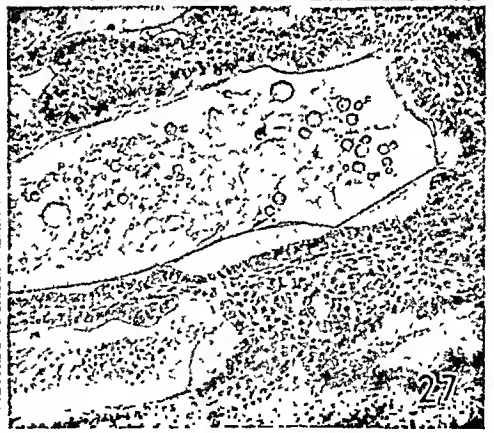
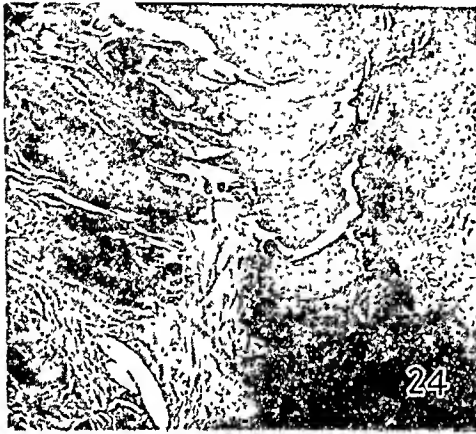


Fig. 22.—A part of the squamous epithelium which lines the wall of a supra-sellar cyst (archeoblastoma). Some of the cells (marked by an arrow) are clumped in the basal part of the epithelium next to the connective tissue membrane. This membrane shows lymphoid cell infiltration; hematoxylin and eosin; $\times 28$.

Fig. 23.—Higher magnification of the cornified cells that have been displaced into the connective tissue and surrounded by giant cells; hematoxylin and eosin; $\times 57$.

Fig. 24.—A more advanced stage of the process illustrated in figures 22 and 23; hematoxylin and eosin; $\times 28$. The cornified masses, still showing cellular structures, have undergone calcification. Close to them is incompletely formed bony tissue.

Fig. 25.—A miscellaneous disposition of some cornified masses with their characteristic structures, others in different periods of calcification, and bony trabeculae completely formed; hematoxylin and eosin; $\times 28$.

Fig. 26.—Authentic adamantinoma; hematoxylin and eosin; $\times 28$. Note the interepithelial spaces where the characteristic hyaline ribbons may be seen, some undergoing calcification. They represent the specific secreting activity of the columnar cells and thus manifest the ameloblastic character of these cells.

Fig. 27.—Adamantinoma. It can be identified by the hyaline ribbons and clumps undergoing calcification; hematoxylin and eosin; $\times 57$.

for classifying the tumor as an adamantinoma. In my opinion, adamantinoma is an epithelial tumor whose peripheral cells, densely arranged, are columnar in shape and secrete a calcifiable albuminous mass. The loose central parts of the epithelial cords of the tumor, to which so many investigators have given attention, may also exist in a simple epithelioma or be missing in an adamantinoma, without giving a clue to the diagnosis.

In figure 26, for example, an authentic adamantinoma is shown. The epithelial masses of the tumor are formed by loose central portions and dense peripheral portions; but the most important detail is to be found in the interepithelial spaces, where characteristic hyaline ribbons are seen, some of them undergoing calcification; they represent the specific secreting activity of the columnar cells, thus manifesting the ameloblastic character of these cells.

In figure 27 there are no loose portions, but the ameloblastic nature of the tumor is made sufficiently apparent by the presence of hyaline ribbons and clumps in the process of calcification.

A similar limitation applies to the pituitary teratoma. Under this heading tumors have been described which are really chromophobe adenoma with choristoblastic inclusions, as the one shown in figure 16, or else ossifying archeoblastoma. I believe that the name "teratoma" should be reserved for those organoid tumors constituted by adult tissues, which are formed from at least two different blastodermic layers; this happens when such tissues exert reciprocal inductions and, therefore, grow synergically. If, as often happens in the pituitary gland, the tumor seems to be derived from a single anlage with multiple potentialities of differentiation, one of which has preponderant development, one is dealing with a hamartoma, using the adequate nomenclature of Albrecht, and not with a true teratoma.

CONCLUSIONS

1. The acidophilic cells of pituitary adenoma lose their capacity for elaborating specific granulations with relative frequency; in this way oligochromic adenoma arises. These tumors tend to adopt one of the following three forms: (1) The tumor cells become spheroid, their nuclei are eccentric in position and lobulated in shape, and they secrete a very fluid albuminous substance, which is deposited in the stroma (exhausted acidophilic adenoma); (2) the tumor cells double their size, show an eccentric nucleus and are arranged in anastomosing cords (megaloeytic adenoma); (3) the tumor elaborates an abundant quantity of colloid substance in which the cells are deposited, forming "isogenic groups" (grouped cell adenoma).

2. Between the typical acidophilic adenoma and the exhausted one, different transitional types are found. The megalocytic adenoma probably represents a frustrated form of acidophilic adenoma. Grouped cell adenoma may be interpreted as a degenerated form of acidophilic adenoma.

3. The polymorphism of the adenoepithelioma of the pituitary gland is a consequence of the multipotentiality of the embryonic epithelium lining the pituitary vesicle. The less differentiated tumors are formed by columnar cells arranged in rows; the most differentiated ones, by polyhedral cells grouped in cords. The chromophobe adenoma represents an intermediate stage between the two extremes.

4. The name "archeoblastoma" has been proposed for the solitary suprasellar cyst, which is limited by a cornified squamous epithelium and contains an aqueous fluid rich in cholesterol crystals and blood pigments. This cyst is a choristoblastic formation which represents persistence of the pituitary vesicle of the embryo in the adult. The most characteristic morphologic feature of the structure of archeoblastoma is the existence of cornified masses embedded in the connective tissue; these masses are first calcified and finally transformed into bony laminae; this is a rare case of ossification arising on epithelial tissue.

5. Only those epithelial tumors which show a capacity for elaborating calcifiable hyaline substance should be considered as adamantinoma. True adamantinoma of the pituitary gland is frequent.

6. The tumors formed from miscellaneous structures derived from a unique and multipotent embryonal anlage are not to be considered as true teratomas. These false teratomas, which are recognizable through the observation that the development of one structure is predominant in comparison with the rest, should be classified under the classic nomenclature of Albrecht as hamartoma when the neoplastic structures belong to the tissue of the normal organ and as choristoma when the tumoral components are foreign to the organ in which they develop. True teratoma of the pituitary gland is rare.

EFFECT OF SODIUM CHLORIDE DEPRIVATION ON THE GROWING RAT

JOHN T. CUTTINO, M.D.

A. S. PARIS, M.D.

AND

MACEY H. ROSENTHAL, M.D.

DURHAM, N. C.

SODIUM and chloride deficiency in experimental animals has received only slight attention. In 1937 Orent-Keiles, Robinson and McCollum¹ reported the gross changes occurring in a group of rats which had been fed a diet deficient in sodium and chloride as a control for their study of sodium deprivation. These changes consisted of bleeding from the mouth, ears, nose and forepaws and passage of dark red urine. At autopsy these rats had hemorrhages in the liver, and the adrenal glands were small but grossly normal. Histologic examination was not reported.

In contrast with the paucity of reports on combined sodium and chloride deficiencies, single element studies on the influence of sodium deprivation and chloride deprivation have been relatively numerous.

For many years sodium deprivation has been known to affect unfavorably both growth and the utilization of nutritive elements.² In addition, sodium lack has been shown by Orent-Keiles, Robinson and McCollum¹ to produce ulceration and keratinization of the cornea, with the formation of an exudate. In addition, a pronounced loss of hair of the eyelids has been recorded. These changes did not respond to administration of carotene. In a subsequent publication Follis, Orent-Keiles and McCollum³ described the mechanism of the ocular change as a caking of the exudate on the lids producing obstruction of the ducts of the meibomian glands. This was followed by metaplasia of the columnar and goblet cells producing keratinization. That injury of cells had been produced was indicated by the fact that the zone was

From the Department of Pathology, Duke University School of Medicine.

1. Orent-Keiles, E.; Robinson, A., and McCollum, E. V.: *Am. J. Physiol.* **119**:651, 1937.

2. (a) Kahlenberg, O. J.; Black, A., and Forbes, E. B.: *J. Nutrition* **13**:97, 1937. (b) Marquis, M.: *Compt. rend. Soc. de biol.* **128**:449, 1938. (c) Orent-Keiles and co-workers.¹

3. Follis, R. H.; Orent-Keiles, E., and McCollum, E. V.: *Arch. Path.* **33**:504, 1942.

infiltrated by polymorphonuclear leukocytes. Retardation of growth of bone and atrophic testicular changes were ascribed to inanition.

In 1942 Voris and Thacker⁴ concluded that the substitution of bicarbonate for chloride in the diet of growing rats was followed by depression of appetite, increased consumption of water, retardation of growth, smaller gain of fat and water with larger proportionate gains of protein and residual organic substance, slight decrease of fecal nitrogen and slight increase of energy utilization with heat production. Greenberg and Cuthbertson⁵ failed to confirm the loss of appetite; they found that the chloride-deficient rats ate more, though they gained less. They found in addition that, as would be expected, the blood chloride was diminished, the carbon dioxide-combining power was increased, and chloride retention occurred within a few hours. Later they⁶ described cystlike changes of the glomeruli and degenerative lesions of the tubules with homogeneous masses in the lumens. Some tubules showed scarring. These changes were ascribed to "overwork of the kidneys in conserving chloride." Lowenhaupt and Greenberg⁷ carried the study further and concluded that calcium was precipitated in the lumens of collecting tubules, with consequent blocking of the tubules and the production of hydronephrosis and a granulomatous reaction in association with foreign material.

Our interest in sodium chloride deficiency has arisen out of a projected study of heat stroke. Inasmuch as heat stroke is considered a hemorrhagic disease based on increased capillary porosity⁸ and inasmuch as hemorrhagic tendencies have been reported in rats deficient in sodium chloride,¹ it seemed likely that if a condition analogous to heat stroke could be produced in rats, it would be facilitated by a dietary sodium and chloride deficiency. Consequently, a pilot experiment was undertaken, in which sodium and chloride deficiency was studied. Our results are at such variance with the findings of others that a separate report of this experiment seems warranted.

MATERIALS AND METHODS

Ninety recently weaned white rats weighing between 21.5 and 59.4 Gm., an average of 35.15 Gm., were fed synthetic diets (table) according to the following regimen: Eighteen were fed a control diet; 72, a diet deficient in sodium and chloride. At the end of one month the test group was divided into three subgroups, and the diets were altered as follows: A group of 24 rats continued to subsist on the sodium and chloride-deficient diet, a group of 24 were fed a sodium-deficient diet—this change being effected by the addition of potassium chloride—and 24 were

4. Voris, L., and Thacker, E. J.: *J. Nutrition* **23**:365, 1942.

5. Greenberg, D. M., and Cuthbertson, E. M.: *J. Biol. Chem.* **145**:179, 1942.

6. Cuthbertson, E. M., and Greenberg, D. M.: *J. Biol. Chem.* **160**:83, 1945.

7. Lowenhaupt, E., and Greenberg, D. M.: *Arch. Path.* **42**:49, 1946.

8. Wright, D. O.; Reppert, L. B., and Cuttino, J. T.: *Arch. Int. Med.* **77**:27, 1946.

given a chloride-deficient diet—effected by the addition of sodium bicarbonate. These changes of dietary regimen were made in an effort to reproduce the changes described for a single element deficiency. A short time before the end of the experiment 3 rats from the chloride-deficient group and 4 rats from the group deficient in sodium and chloride were transferred to the control diet in order to observe the effect on the growth of the rat following this restoration of sodium chloride. The rats were housed in cages containing groups of 6. Because of a tendency toward cannibalism among rats, it was necessary to drop 41 rats from the experiment to avoid the possibility of error of interpretation from this source. Food was weighed each day to ascertain that adequate intake of food was maintained, and 2 animals were killed at weekly intervals beginning at the fourth week.

GROSS APPEARANCE OF THE ANIMAL

The weight curve showed a distinct retardation of growth in sodium and chloride-deficient animals (fig. 1). On separation into sodium and chloride-deficient,

Formulas of Diets

	Control	Sodium and Chloride- Deficient Diet	Sodium- Deficient Diet	Chloride- Deficient Diet
Sucrose.....	1,300 Gm.	1,300 Gm.	1,300 Gm.	1,300 Gm.
Caseln.....	400 Gm.	400 Gm.	400 Gm.	400 Gm.
Hydrogenated cottonseed oil ("Crisco").....	200 Gm.	200 Gm.	200 Gm.	200 Gm.
Choline di-hydrogen citrate.....	4 Gm.	4 Gm.	4 Gm.	4 Gm.
Vitamin mixture *.....	½ unit	½ unit	½ unit	½ unit
Basic salt mixture †.....	102.5 Gm.	102.5 Gm.	102.5 Gm.	102.5 Gm.
Sodium chloride.....	20 Gm.
Potassium chloride.....	15 Gm.
Sodium bicarbonate.....	34 Gm.

* The vitamin mixture was made up of: percomorph liver oil, 4 Gm.; thiamine hydrochloride, 10 mg.; riboflavin, 20 mg.; pyridoxine hydrochloride, 10 mg.; calcium pantothenate, 100 mg.; nicotinic acid, 30 mg.; wheat germ oil, 4 mg., and naphthohydroquinone, 50 mg. Note: This formula is considered 1 unit and added to 4 Kg. of diet.

† The basic salt mixture was made up of: CaCO_3 , 300 Gm.; $\text{K}_2\text{CO}_3 \cdot 1\frac{1}{2} \text{H}_2\text{O}$, 220 Gm.; KH_2PO_4 , 340 Gm.; MgO , 40 Gm.; $\text{FeC}_6\text{H}_5\text{O}_7 \cdot 3\text{H}_2\text{O}$, 100 Gm.; CuSO_4 , 20 Gm.; MnSO_4 , 5 Gm.

chloride-deficient and sodium-deficient groups, the growth curve continued low. The chloride deficient group grew slightly faster than the sodium and chloride-deficient group, and the sodium-deficient group did not grow as fast as the group deficient in sodium and chloride. These findings are in essential agreement with previous observations.⁹ When rats previously fed sodium-deficient and sodium and chloride-deficient diets were changed to the control diet, there was rapid acceleration of growth. This agrees with previous observations.^{2b} As in previous reports,¹⁰ alterations occurred in the conjunctiva at about the eighth to tenth week. These changes were grossly most numerous in the sodium and chloride-deficient group (40 per cent) and the sodium-deficient group (36.3 per cent), but they also occurred in the chloride-deficient group (12 per cent) and the control (14.3 per cent). There

9. Orent-Keiles and co-workers.¹ Kahlenberg and co-workers.^{2a} Marquis.^{2b} Follis and co-workers.³ Voris and Thacker.⁴ Greenberg and Cuthbertson.⁵

10. Orent-Keiles and co-workers.¹ Kahlenberg and co-workers.^{2a} Marquis.^{2b} Follis and co-workers.³

was loss of hair on the shoulders and the head in 20 per cent of the sodium and chloride-deficient group and 9 per cent of the sodium-deficient group. This feature was not observed in the control or in the chloride-deficient group.

MICROSCOPIC OBSERVATIONS

Sections of liver, spleen, pancreas, kidney, skin, stomach, intestine, lung, heart, thyroid gland and trachea, tongue, eye, brain, bone, testicle and adrenal gland were routinely examined. Hematoxylin and eosin preparations were used routinely and special technics as required, such as von Kossa stains for calcium identification.

Sodium-Deficient Group.—Atrophy of the epithelium of the conjunctiva and meibomian ducts was observed in 25 per cent of the 12 animals of this group which were considered valid for the experiment. Polymorphonuclear leukocytes were observed infiltrating the conjunctivas in only 16.6 per cent of these animals (fig. 2). This reaction is similar to that described by Orent-Keiles and co-workers¹ and Follis and co-workers.³ However, in this series it is to be pointed out that this

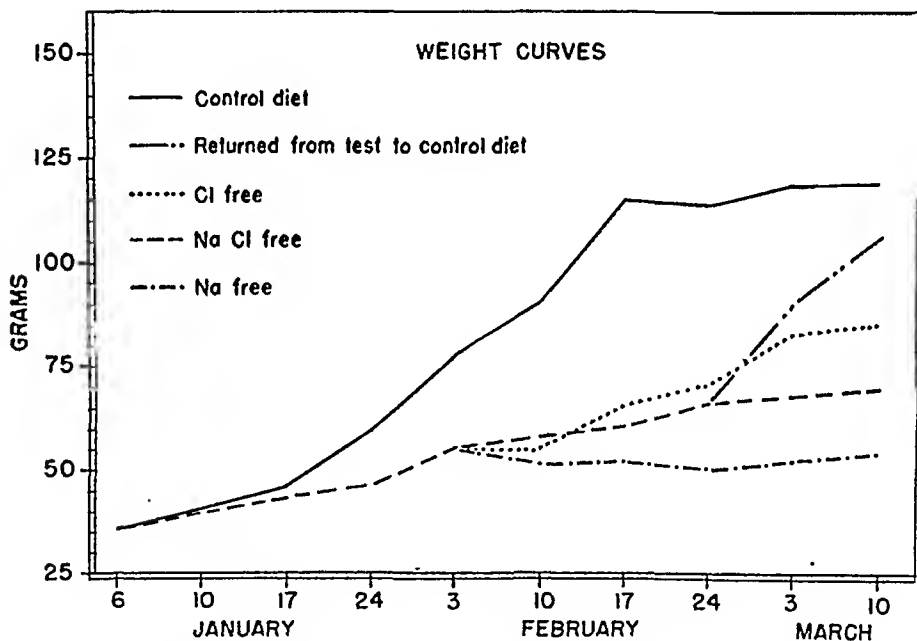


Fig. 1.—Weight curves showing retardation of growth observed as a result of variation in diet; a marked acceleration of weight gain is noted in the group of rats returned from a test diet to the control diet.

reaction was not specific and was found in all categories of animals. Deposition of calcium was noted in the muscle in 16.6 per cent and in the brain in 8.3 per cent (fig. 3 A). In both the muscle and the brain the initial injury was focal necrosis with calcification and cellular reaction in the form of lymphocytic infiltration and macrophagic proliferation. These lesions were also surrounded by glial (astrocytic) proliferation. Two of the animals had pneumonic consolidation and renal hemorrhage with dilatation of the renal tubules. The bones uniformly showed retardation of growth at the epiphysial line.

Chloride-Deficient Group.—Calcium deposition similar to that described by Lowenhaupt and Greenberg⁷ was observed in the tubular epithelium of the kidney in 12.5 per cent of the 8 animals of this group which were considered valid (fig.

3 *B* and *C*). However, this same process was found in 23.6 per cent of the sodium and chloride-deficient animals and 14.3 per cent of the control animals. However, mononuclear cell infiltration of the interstitial tissues of the kidneys was found in 37.5 per cent. This type of lesion was found in only 1 other animal, and this



Fig. 2.—*A*, atrophy and inflammation of the conjunctival sac, found in sodium-deficient and sodium and chloride-deficient rats and, less frequently, in chloride-deficient rats. Hematoxylin and eosin; $\times 350$. *B*, atrophy and inflammation of lacrimal ducts. Hematoxylin and eosin; $\times 150$.

animal belonged to the sodium and chloride-deficient group. We were unable to confirm the report of dilatation of tubules and hydronephrosis observed in connection with this type of lesion. The focal necrosis with deposition of calcium was found in the brain in 12.5 per cent of this test group. There was atrophic change

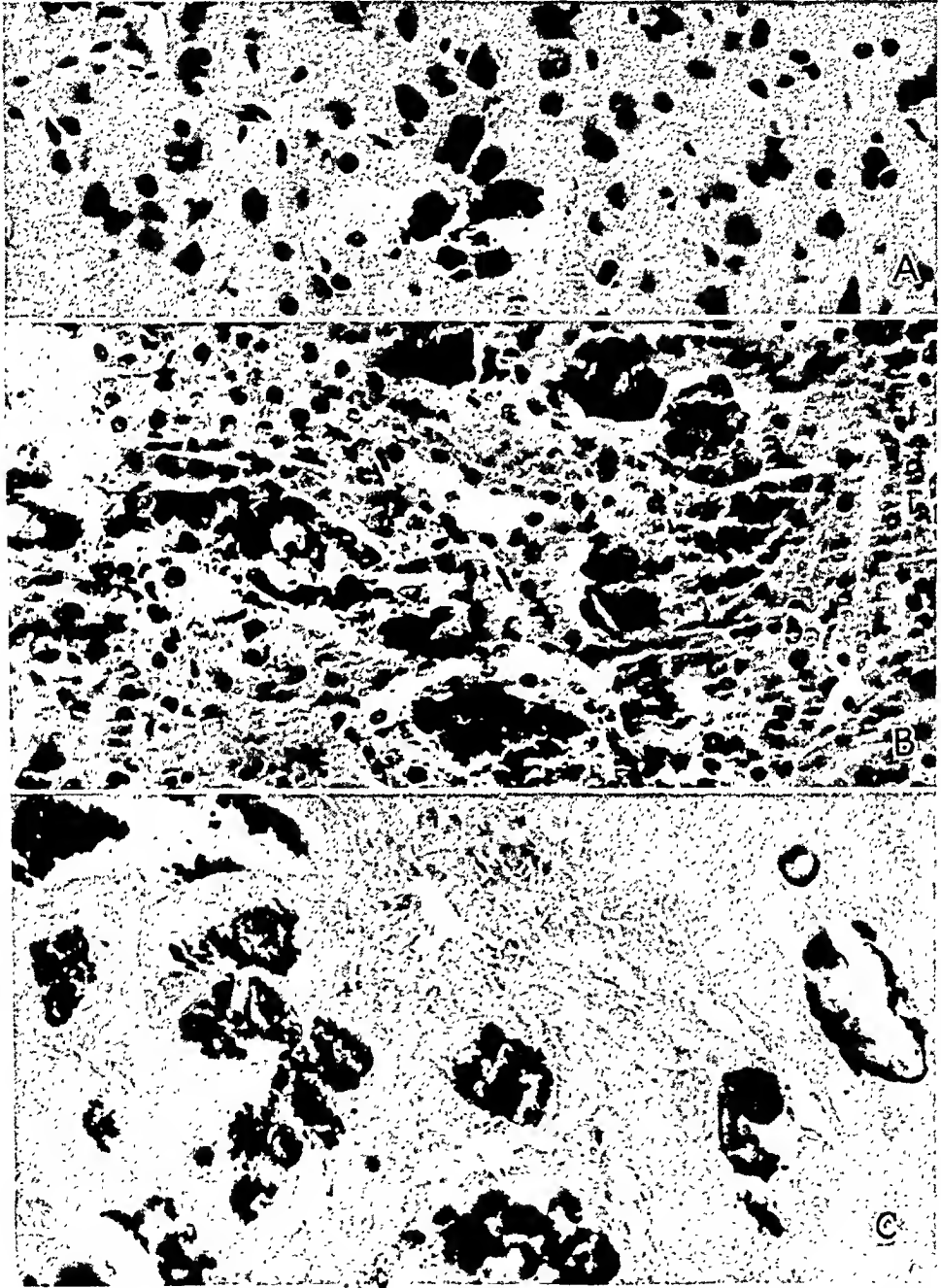


Fig. 3.—*A*, calcium deposited in the brain, in an area of gliosis; the specificity of this lesion is not apparent, but it occurs in greatest frequency in the chloride-deficient and sodium and chloride-deficient groups. Hematoxylin and eosin; $\times 350$. *B*, calcium deposition in renal tubules. No dilatation of tubules was found. Hematoxylin and eosin; $\times 350$. *C*, another view of calcification of renal tubules. Von Kossa stain; $\times 350$.

of the conjunctiva with cellular infiltration in 18.1 per cent and atrophy of the lingual epithelium in 39.5 per cent. One instance each of edema of the lung, hemorrhage of the kidney and necrosis of the liver was observed.

Sodium and Chloride-Deficient Group.—Atrophy of the conjunctival epithelium similar to that found in sodium deficiency was observed in 64.7 per cent of the 17 animals of this group which were considered valid. There was cellular infiltration, chiefly of polymorphonuclear leukocytes, in the conjunctiva and subconjunctiva in 53 per cent. There was atrophy of the skin in 20 per cent and atrophy of the tongue in 10 per cent. Atrophy of the testicular epithelium was observed in 15 per cent. Calcium deposition was found in the renal tubular epithelium in 23.6 per cent and in the brain in 10 per cent. Pneumonic consolidation was found in 33 per cent and pulmonary hemorrhage in 5 per cent.

Animals Returned to Control Diet.—The animals returned from test diets to the control diet showed accentuation of the lesions as indicated by frequency of occurrence. Of the 3 rats from the chloride-deficient group fed the control diet, 2 had calcium deposits in the renal tubular epithelium and also the focal lesion with calcification in the brain. One showed atrophy of the conjunctiva, with polymorphonuclear leukocytes infiltrating the stratified squamous epithelium and subepithelial tissues. Of the 4 rats returned to the control diet from the sodium and chloride-deficient diet, all 4 showed atrophy of the conjunctiva, and 2 had polymorphonuclear leukocytes infiltrating the tissues. Three of the 4 rats showed degenerative changes in the voluntary muscles with macrophagic response and calcification and calcium deposition in the renal tubular epithelium. There was 1 instance each of calcium deposition of the cerebral cortex, atrophy of the skin and atrophy of the testis.

COMMENT

In analyzing the results of our experiment we have been able to recognize the lesions described in single element deficiencies. However, we have been unable to attain any degree of specificity in the production of the lesions. The ocular changes described in connection with sodium deficiency have been produced in our experiment in all types of deficiency. The animals deficient in sodium and chloride are even more prone to show these changes than are the sodium-deficient animals. The hypothesis advanced by Follis,¹¹ that the ocular changes in sodium-deficient rats might be explained on the basis of the production of electrolyte-reduced tears with consequent lowering of concentration to the point of isotonicity or even hypotonicity, a state producing irritation of the conjunctival epithelium, which is conditioned to hypertonicity, seems to us equally applicable to sodium and chloride deficiency, as well as to chloride deficiency. On the other hand, it might also be postulated that a deficiency of electrolytes diminishes the secretion of tears, producing irritation by simple drying.

The deposition of calcium in the renal tubules was an inconsistent and nonspecific findings in all test groups. In only 1 instance in the whole of the experiment were we able to demonstrate the dilatation of the tubules described in chloride deficiency. The pathogenesis of the

11. Follis, R. H.: *The Pathology of Nutritional Disease*, Springfield, Ill., Charles C Thomas, Publisher, 1948.

deposition of the calcium of the tubules may well be explained by the mechanism proposed by Lowenhaupt and Greenberg; that is, owing to the alkalinity of the urine with possible elevation of phosphate concentration in convoluted and collecting tubules, the tubular epithelium is damaged and favorable circumstances are provided for the precipitation of calcium salt. However, we were not able to confirm the finding that this precipitation produced an obstruction of the lumens of these tubules with consequent development of hydronephrosis, since in only 1 animal were we able to make such an observation. The significance of this observation therefore seems entirely compatible with the finding of nonspecificity of this lesion, in that one might expect to find this type of alteration in any of the deficiencies studied by us. In addition to the deposition of calcium salt in the tubules of the kidney, we were able to find deposition of this salt in muscles and brain. In the brain the lesion consisted of deposition of calcium salt about damaged nerve cells of the cortex as indicated by von Kossa's stain, and with this injury there appeared a reaction in the form of gliosis. These lesions were found in greater numbers among the chloride-deficient animals, but the observation could be made in all of the test animals, with the least consistent findings being recorded for the sodium and chloride-deficient group. Other pathologic conditions, such as atrophy of the tongue, edema of the lungs, necrosis of the liver, and pneumonia with abscess formation, were found without regard to specificity in the test groups and without sufficient consistency to warrant interpretation.

We were unable to find any evidence of hemorrhagic tendencies in any of these groups, including the group fed the diet deficient in sodium and chloride. It might be that the discoloration observed by Orent-Keiles, Robinson and McCollum was due to production of porphyrin and not to blood.

SUMMARY

Sodium and chloride, chloride, and sodium deficiencies were studied in colonies of growing rats by means of synthetic diets.

We have been unable to determine or recognize hemorrhagic tendencies in any of these deficiencies.

Changes in the conjunctiva and meibomian ducts similar to those described for sodium deficiency were found in the animals of this experiment. We were unable to find a specificity for this lesion since it was observed with equal frequency in the sodium and chloride-deficient group and the sodium-deficient group, and was noted in 3 rats of the chloride-deficient group.

Calcium deposition in the renal tubules similar to that described in connection with chlorine deficiency was found in these test groups, but without consistency or specificity. We were unable to confirm the findings of hydronephrosis.

General Reviews

INTERRELATIONSHIP OF DISEASES OF THE LIVER AND THE BRAIN

A. B. BAKER, M.D.

MINNEAPOLIS

WITH the increasing interest in specialization, more and more research is being focused on diseases of isolated organs. As a result of this trend it is frequently forgotten that there is often an interrelationship between the diseases of the various body structures. One of the most striking and as yet unsolved examples of such associated organic diseases is the combined involvement of the liver and the brain. This unusual association of diseases of separate organs was first described about sixty years ago by Gowers,¹ who observed 2 cases in which acute chorea was associated with cirrhosis of the liver. At autopsy, however, no lesions were observed within the central nervous system. Two years later, in 1890, Ormerod² and Homén³ described in separate publications the appearance of cerebral and hepatic disease as concomitant occurrences. Ormerod reported a single case of acute involvement in which a boy at autopsy had bilateral symmetric softening of the putamen and cirrhosis of the liver. Homén described a unique and unknown chronic familial disease affecting 3 of 11 children and marked by progressive chorea, dementia, and death in three to seven years. All the patients (two brothers and a sister) showed symmetric lenticular lesions and severe cirrhosis of the liver.

In 1912 Wilson,⁴ in a masterful publication, described in detail a rare familial disease of the nervous system which he called "progressive lenticular degeneration" and which has since been known as Wilson's disease or, more recently, as hepatolenticular degeneration (Hall⁵). This disease was characterized by the progressive development in young adults of widespread involuntary movements (usually in the nature of

From the Division of Neurology, University of Minnesota Medical School.

These studies were aided by a grant from the John and Mary R. Markle Fund.

1. Gowers: A Manual of Diseases of the Nervous System, London, J. & A. Churchill, 1888, vol. 2, p. 656.

2. Ormerod: St. Barth. Hosp. Rep. 26:57, 1890.

3. Homén, E. A.: Neurol. Centralbl. 9:514, 1890.

4. Wilson, S. A. K.: Brain 34:295, 1912.

5. Hall, H. C.: La dégénérescence hepatolenticulaire, Paris, Masson & Cie, 1921.

tremor or chorea), severe rigidity of the musculature, difficulty in articulation and deglutition, and contractures with progressive emaciation. There was also some associated emotionalism, such as spasmodic crying and laughing, and a slight degree of dementia. The course was acute or chronic and invariably terminated fatally. Pathologically, the most striking lesions consisted of bilateral symmetric degeneration of the lenticular and caudate nuclei and cirrhosis of the liver.

The most striking of the clinical features of Wilson's disease are the tremor and the rigidity. The tremor often appears first in the upper limbs and is rhythmic, often wide in range, and severe. It soon spreads to involve all the skeletal musculature, especially the distal groups, but may also implicate the jaw, the head and the neck. Concomitant with, or shortly after, the onset of the tremor the muscular rigidity makes its appearance. The rigidity of the limbs and the face becomes progressive and outstanding. As a result of the muscular stiffness, there is considerable difficulty in maintaining equilibrium. Helplessness becomes more advanced and even profound. Contractures appear at the elbow, the knee and the hip. The facial musculature also becomes rigid and immobile; the corners of the mouth become retracted, and the upper teeth and gums remain visible, producing the appearance of a rather fatuous persistent smile. Saliva often runs from the lips. Dysphagia and dysarthria soon appear. The difficulty of articulation becomes progressive until the patient is unable to speak at all (this adding to his helplessness). The dysphagia produces progressive emaciation, which is a factor in the lethal outcome. At no time do these patients show any true weakness or sensory involvement.

Mental changes are generally more apparent than real. The patient's extreme rigidity, the fixed smile and the dysarthria often produce a false impression of the patient's mental status; however, definite emotional disturbances do occur. There is emotional overaction with involuntary laughing or crying and some narrowing of the mental sphere. As a rule the intellectual faculties are well preserved in spite of the "idiotic" appearance of the patient. Campbell and Morse⁶ observed some intellectual impairment in their patients.

Another curious feature of this disease is the occasional presence of a ring of greenish brown pigment on the under surface of the cornea near the limbus. This is known as the Kayser-Fleischer,⁷ ring and when present is characteristic of this symptom complex.

Generally the cirrhotic liver produces no clinical disturbances. As a rule, the liver is not palpable and tests of function have proved incon-

6. Campbell, C. M., and Morse, M. E.: *J. Neurol. & Psychopath.* **5**:28, 1924.

7. Kayser, B.: *Klin. Monatsbl. f. Augenh.* **40**:22, 1902. Fleischer, B.: *Deutsche Ztschr. f. Nerven.* **44**:179, 1912.

clusive with respect to hepatic dysfunction. In 3 of Wilson's cases an attack of jaundice preceded the cerebral symptoms by years. Apparently initial jaundice and even ascites are not uncommon as predecessors of the illness, but actual symptoms of cirrhosis appearing during the progression of the cerebral symptoms are uncommon. As will be shown later, many authors feel that the hepatitis may be fatal before cerebral symptoms appear. Cases have been described in which such isolated hepatic disease has occurred in siblings of patients suffering from typical Wilson's disease.

Clinically, two types of the disease can be recognized, the acute and the chronic. In the acute form there is often a slight elevation of temperature with a fatal outcome in a few months (de Lisi,⁸ Howard and Royce⁹ and von Dziembowski¹⁰). In the chronic form the course of the illness may extend over a period of many years (forty-three years, according to Lüthy¹¹). Cases have been reported from many parts of the world. There is no sex prevalence.

The pathologic aspects of this disease are also very unusual and characteristic. There is generally bilateral and symmetric involvement of the lenticular nucleus implicating chiefly the putamen and to a lesser extent the globus pallidus, the caudate nucleus and the external capsule. In many cases the involved structures are shrunken and sclerosed, while in other cases these structures show softening necrosis and cavity formation. Histologically, there is a gradual destruction of parenchyma, both cells and processes, with these being gradually replaced by a tremendous glial overgrowth. During the process of degeneration numerous fat granule cells invade the involved areas. The macroglial overgrowth results in the formation of numerous giant glial cells referred to as Alzheimer cells. If the process becomes arrested at this stage, the involved structures appear shrunken. Often, however, the glial overgrowth also undergoes degeneration resulting in the formation of cystic cavities filled with a loose meshwork of glial fibers and remnants of the preceding degenerative process. No inflammatory elements can be seen. Scattered lesions can also be found in other regions of the nervous system, such as the optic thalamus, the cerebral cortex, the red nucleus and the cerebellum. These consist of mild nerve cell changes and some glial reaction (Pollak,¹² Pfeiffer¹³ and Howard and Royce⁹). Actually, as one reviews the literature, it becomes more and more apparent that the cerebral lesions are not localized to the lenticular nuclei as emphasized by Wilson, but occur with equal frequency and severity within

8. de Lisi, L.: *Riv. di pat. nerv.* **34**:1, 1929.

9. Howard, C. P., and Royce, C. E.: *Arch. Int. Med.* **24**:497, 1919.

10. von Dziembowski, S.: *Deutsche Ztschr. f. Nervenhe.* **57**:295, 1917.

11. Lüthy, F.: *Deutsche Ztschr. f. Nervenhe.* **123**:101, 1932.

12. Pollak, E.: *Ztschr. f. d. ges. Neurol. u. Psychiat.* **77**:37, 1922.

13. Pfeiffer, J. A. F.: *J. Nerv. & Ment. Dis.* **65**:289, 1917.

scattered areas of the cerebral cortex and subcortical white matter (Barnes and Hurst,¹⁴ von Braunmühl,¹⁵ Bielschowsky and Hallervorden¹⁶ and Richter¹⁷). The hemispheric lesions resemble those within the lenticular nucleus and consist of a breakdown of the interstitial tissues with secondary reaction of the neuroglia. In many cases there is a frank, grossly visible necrotizing destruction of the cerebral convolutions, while in others only microscopic changes are seen, consisting chiefly of degeneration of the neurons and glia without neuroglial proliferation or vascular changes (Eicke,¹⁸ Kuiper¹⁹ and Spielmeyer²⁰).

The liver always shows an advanced stage of portal cirrhosis. It is generally smaller than normal, firm, hard and nodular. In some nodules scarcely a single normal cell is found, most of the cells being necrotic and shrunken. There is marked proliferation of the portal connective tissue, which is filled with hypertrophying bile ducts.

From the characteristic features of this syndrome it is apparent that the pathologic process may involve predominantly the liver, the brain or the cornea. Obviously, any combination of these involvements may occur. In typical cases of Wilson's disease the predominant alterations appear within the extrapyramidal system and the liver. Since the cerebral manifestations are so striking, these cases are readily recognized; however, less typical cases occur in which there is predominant involvement of the liver or the cornea and hence may be entirely overlooked. For example, Lhermitte and Muncie,²¹ reported 3 cases in which the patients were members of a single family in which the illness manifested itself chiefly as cirrhosis of the liver with actual hepatic dysfunction (urobilin and urobilinogen in urine, direct and delayed reactions in the direct van der Bergh test, icterus index 28.5, increased blood cholesterol). One of these patients had a typical Kayser-Fleischer ring. In none was there active evidence of involvement of the nervous system; however, on careful scrutiny there was noticed clumsiness of the hands, mild tremors of the upper extremities and slight incoordination of the limbs. Barnes and Hurst¹⁴ studied a family in which cirrhosis of the liver developed in 4 members, and only 1 showed a Kayser-Fleischer ring. Two of these patients presented the typical appearance of Wilson's disease, while 2 showed no nervous manifestations. Histologically,

14. Barnes, S., and Hurst, E. W.: *Brain* **48**:280, 1925.

15. von Braunmühl, A.: *Ztschr. f. d. ges. Neurol. u. Psychiat.* **130**:1, 1930.

16. Bielschowsky, M., and Hallervorden, J.: *J. f. Psychiat. u. Neurol.* **42**: 177, 1931.

17. Richter, R.: *J. Neuropath. & Exper. Neurol.* **7**:1, 1948.

18. Eicke, W. J.: *Arch. f. Psychiat.* **114**:214, 1941.

19. Kuiper, F. C.: *Over Haemachromatosis met Hepato-cerebral Degeneratie*, Haarlem, J. Enschedé, 1932.

20. Spielmeyer, W.: *Ztschr. f. d. ges. Neurol. u. Psychiat.* **57**:312, 1920.

21. Lhermitte, J., and Muncie, W. S.: *Arch. Neurol. & Psychiat.* **23**:750, 1923.

in spite of the paucity of the clinical symptoms, lesions were found consistently within the lenticular nuclei and consisted of marked nerve cell damage associated with both progressive and regressive neuroglial alterations. Nerve cell changes also occurred within the cerebral cortex.

For some time there has been a great deal of confusion in the literature regarding the relationship of Westphal-Strümpell pseudosclerosis and Wilson's disease. Pseudosclerosis was first described by Westphal²² in 1883, to include 2 cases in which the finding vaguely resembled the syndrome later described by Wilson. The patients had tremors of the limbs, rigidity, disturbance of speech and even attacks of unconsciousness. In none was the condition of the liver described. In 1899 Strümpell²³ revived this term to describe a condition observed in 3 patients, 2 of whom were syphilitic. None of Strümpell's cases resembled cases of hepatolenticular degeneration. In 1902, and later in 1912, Fleischer⁷ described a greenish pigmentation of the cornea occurring in a patient with cirrhosis of the liver. His later paper, entitled "On a Hitherto Unknown Disease Related to Pseudosclerosis," includes for the first time the symptom complex of muscular rigidity, tremor, dysarthria and dementia, as well as corneal pigmentation. It is this paper that really gave impetus to the term "pseudosclerosis," which has been adhered to consistently, chiefly by the German writers. Actually, according to present definition, cases of pseudosclerosis are identical with cases of Wilson's disease; and it might be best to adopt the more descriptive term "hepatolenticular degeneration" originated by Hall⁵ for both diseases.

The etiologic factors of hepatolenticular disease are quite obscure, but a brief review of possible etiologic factors might throw some light on this unusual liver-brain relationship. The following theories as to causation have been proposed:

1. That this disease is a hereditodegenerative process affecting both organs.
2. That the condition is acquired and the two organs are injured concomitantly by some endogenous toxin (Sjövall,²⁴ Löwy²⁵ and Braunmühl¹⁶).
3. That the condition is acquired but that the chief injury is to the liver, with the cerebral lesions being secondary to the hepatic injury (Kehrer,²⁶ de Lisi,⁸ Pollak,¹² Brückner,²⁷ Weger and Natanson²⁸ and Lüthy¹¹).

22. Westphal, C.: *Arch. f. Psychiat.* **14**:87, 1883.

23. Strümpell, A.: *Ztschr. f. Nervenhe.* **16**:348, 1899.

24. Sjövall, E.: *Acta path. Scandinav.* **6**:193, 1929.

25. Löwy, J.: *Deutsches Arch. f. klin. Med.* **141**:213, 1922.

26. Kehrer, F.: *Ztschr. f. d. ges. Neurol. u. Psychiat.* **100**:476, 1926.

27. Brückner: *Jahrb. f. Kinderh.* **110**:284, 1925.

28. Weger, A. M., and Natanson, D. M.: *Arch. f. Psychiat.* **88**:598, 1928.

4. That the condition is acquired and is secondary to the presence of some exogenous toxin.

Wilson and many other investigators have suggested that this illness may be acquired. They point out that prior to the appearance of the illness the patients and their families are normal physically and mentally. There is generally complete absence of any stigmas, such as congenital malformations, and no evidence of any constitutional defects. Furthermore, in its acuteness the illness is unlike any congenital illness. It would seem from a review of the literature that there still remains the possibility that this illness is some form of hereditodegenerative process in spite of the healthy family history and the rapid course. Certainly, similar sporadic appearances are seen in other conditions assumed at present to be degenerative.

From the etiologic standpoint the one constant feature is the presence of a damaged liver. This has led many investigators to the belief that the hepatic involvement precedes and is in a large part responsible for the cerebral lesions. Barnes and Hurst¹⁴ felt that the primary factor was the hepatic damage. They expressed the belief that the liver became injured through a bacillary infection from the intestinal tract. They concluded that the disease of the liver was not chronic progressive cirrhosis but was the result of a series of attacks of acute hepatitis spaced over weeks or months. Occasionally the severity and the frequency of the hepatitis were sufficient to cause death before any nervous symptoms appeared.

The possibility of an exogenous toxin producing this disease has been emphasized through the work of Edsall and Drinker.²⁹ These investigators found that massive doses of certain metals, chiefly manganese chloride (10 to 60 mg.), produce selective damage in the liver and the brain. The liver shows parenchymatous degeneration amounting in some areas to necrosis. Mella³⁰ observed neuronal degeneration and glial reaction of the lenticular nucleus in monkeys inoculated with this metal.

HEMOCHROMATOSIS

Hemochromatosis is considered a true morbid entity characterized by generalized pigmentation of the skin, severe diabetes and enlargement with cirrhosis of the liver. The term was first used by von Recklinghausen,³¹ in 1889, to indicate a histologic condition in which hemofuscin, as well as hemosiderin, was present in various body organs. Although the aforementioned three features characterize this disease, they do not

29. Edsall, D., and Drinker, C.: *Contributions to Research*, 1:447, 1919.

30. Mella, H.: *Arch. Neurol. & Psychiat.* 11:405, 1924.

31. von Recklinghausen, F.: *Verhandl. d. Versamml. Gesellsch. f. Kinderh. deutsch. Naturf. u. Aerzte* 62:324, 1889.

consistently appear in each case and when present often vary in severity. For example, many cases have been reported in which the diabetes has been absent (Opie³² and Osler³³). The etiologic factors of this condition are still unknown, although Sheldon³⁴ felt that it was congenital and due to a fundamental disorder of the iron metabolism with accumulations of small amounts of pigment over a long period.

The first suggestion that there might be some relationship between hemochromatosis and Wilson's disease appeared in 1940, when Thaddea and Oettel³⁵ described pigmentation of skin and brain in a typical case of Wilson's disease. At autopsy the lenticular nucleus was small and pigmented. The nerve cells were decreased, and the glia was proliferated. The skin over the abdomen and the lower extremities showed increased pigmentation in the basal layers. No mention of diabetes was made in this case.

Even more striking were the cases of Brouwer³⁶ and Waggoner and Malamud.³⁷ Brouwer described a case in which both Wilson's disease and hemochromatosis were present clinically and pathologically. The patient, a 59 year old man, had bronzed skin, diabetes and cirrhosis of the liver. At the same time tremor of the hands developed with rigidity of the musculature, stiffness and difficulty of gait, dysarthria, dysphagia and increased emotionalism. At postmortem examination the usual signs of hemochromatosis were observed. The central nervous system showed parenchymal destruction with cavity formation in both putamens and in the caudate nucleus. Alzheimer cells were visible in scattered areas throughout the brain. Waggoner and Malamud described a classic case of hemochromatosis, but the cerebral symptoms were of a more diffuse nature, consisting of mental confusion, ataxia, hypertonia, facial palsy, dysarthria and reflex changes. At autopsy, aside from the hemochromatosis, there were widespread and severe lesions within the cerebral cortex, the putamen, the corona radiata, the globus pallidus, the red (or tegmental) nucleus, the cerebral peduncles and the cerebellar white matter. The lesions consisted of foci of status spongiosus with little glial reaction. Finally, Lewey and Govons³⁸ and Neumann³⁹

32. Opie, E. L.: *J. Exper. Med.* **4**:279, 1899.

33. Osler, W.: *Brit. M. J.* **2**:1899.

34. Sheldon, F. H.: *Haemochromatosis*, London, Oxford University Press, 1935.

35. Thaddea, S., and Oettel, H. J.: *Ztschr. f. d. ges. Neurol. u. Psychiat.* **170**:552, 1940.

36. Brouwer, B.: *Proc. Roy. Soc. Med.* **29**:579, 1935.

37. Waggoner, R. W., and Malamud, N.: *Tr. Am. Neurol. A.* **67**:45, 1941.

38. Lewey, F. H., and Govons, S. R.: *J. Neuropath. & Exper. Neurol.* **1**:129, 1942.

39. Neumann, M. A.: *J. Neuropath. & Exper. Neurol.* **7**:19, 1948.

reported cases of hemochromatosis of the central nervous system. The patients showed no evidence of diabetes or of cirrhosis of the liver.

We, too, have had the opportunity of studying the nervous system in a typical case of hemochromatosis.

CASE 1.—The patient, a 62 year old man, became ill four years before his admission to the hospital. At the time of his admission there was bluish pigmentation of the hands and forearms, the face and the exposed areas of the neck and there was increased bronzing of the entire skin. Examination revealed the liver to be enlarged and firm. It was palpable 6 cm. below the costal margin. Laboratory examination of the urine showed sugar (4 plus). A dextrose tolerance test established a diagnosis of diabetes.

This patient was followed for four years. On his last admission he was weak, cachectic, incontinent and somewhat disoriented. The abdomen revealed no abnormality except for slight enlargement of the liver. During his hospital stay he became increasingly more stuporous. Neurologic examination showed scattered defects, with the left biceps jerk being greater than the right, but the right knee jerk being greater than the left. Gordon's reflex was present on the right. A lumbar puncture showed clear fluid under normal pressure; there were no cells. The blood urea nitrogen was 5 mg. per hundred cubic centimeters. Despite the treatment, the patient's course was rapidly downhill. Terminal studies of liver function gave essentially normal results.

At autopsy there was generalized bronzing of the skin. The liver weighed 1,835 Gm. Its external surface showed a fine nodular cirrhosis. On cut section the liver was firm and had a homogeneous brownish appearance. Histologic sections of the liver showed severe portal cirrhosis. In many areas the hepatic cells were atrophic and necrotic. External examination of the brain revealed a small amount of subarachnoid bleeding. The basilar vessels appeared normal. The rest of the brain showed nothing on gross examination.

Histologic examination of the brain revealed demyelination, which was chiefly perivascular in distribution, involving the entire cerebral hemispheres. The degree of demyelination was variable around different vessels. In some areas it was mild, with only swelling of the myelin sheaths. Around other vessels the myelin swelling and vacuolation extended for some distance away from the vessel and merged with similar areas around adjacent vessels. There were no fat granule cells in any of the areas of demyelination.

The cerebral cortex showed the same type of perivascular changes. In most areas the cortical involvement was not so extensive as that seen in the white matter and was localized chiefly to the inner cortical layers. The nerve cells appeared to have escaped any alteration with the exception of some of the cells in the inner two cortical layers, particularly those adjacent to areas of perivascular demyelination. Around these areas the nerve cells showed fairly extensive changes, consisting primarily of chromatolysis and absence of the staining properties. Many of the cells appeared to stain lightly, often only the outlines of the neurons being visible. Scattered neurons appeared shrunken, irregular and even fragmented.

The most extensive changes seen in this brain were localized within the hippocampal and calcarine regions. In these areas the white matter appeared to be almost completely destroyed and replaced by diffuse confluent areas of demyelination with actual cyst formation. Practically all the nerve cells within these regions showed destruction. The cells stained poorly, many being irregular and fragmented. In these regions only an occasional intact neuron could be

observed. In the region of the basal nuclei the thalamus appeared to be uninvolved. There was a most extensive destruction of the putamen and the globus pallidus. Here there was striking perivascular demyelination. The nerve cells also were extensively altered. Most of the larger cells were shrunken and stained poorly. In many cells the cytoplasm was fragmented, and that of some cells was entirely absent, leaving an isolated nucleus to indicate the previous presence of a normal nerve cell. Many ghost cells were also visible within the region of the globus pallidus. In the pons the changes were mild. There was definite granular ependymitis of the entire fourth ventricle. Within the pons the demyelination was limited primarily to the perivascular regions. Only occasional nerve cells showed some chromatolysis. No changes were observed within the region of the medulla.

ERYTHROBLASTOSIS FETALIS OR KERNICTERUS

This condition was apparently first described by Orth,⁴⁰ in 1875, at which time he reported a pathologic study of a jaundiced infant who died on the second day of life and showed at autopsy bilirubin crystals in the nuclear masses of the brain. This syndrome was first named kernicterus by Schmorr,⁴¹ in 1902. During the past fifty years there have been many clinical and pathologic reports of this condition in which various neurologic symptoms have been described as complicating neonatal jaundice in infants from normal parents. Generally, the newborn infant becomes jaundiced within a few hours or a few days after birth and exhibits lethargy and drowsiness, together with a slight rise of temperature. Some infants are restless and show excessive crying with some rigidity or spasticity and even convulsive movements. Symptoms such as diarrhea, vomiting, irregular respirations and even circulatory collapse may occur. In many of the cases in which a child survives a milder form of the disease, it shows, after a period of a few weeks or even a few months, manifestations of a chronic neurologic involvement, namely, chorioathetotic movements, ataxia, rigidity, spasticity, convulsions, emotional instability and even mental retardation. Both in the acute and in the subacute or chronic stage the neurologic picture is most variable. The only consistent observation is that this illness occurs in infants who become jaundiced shortly after birth and whose parents are apparently normal.

For a long time the cause of this condition was unknown. It was regarded as an expression of intoxication due to dysfunction of the fetal liver or of the blood-forming organs. Some authors considered it the result of maternal intoxication. Real progress was made in the understanding of the illness by the discovery of the Rh factor by Landsteiner and Wiener.⁴² The discovery of the role played by the

40. Orth, J.: *Virchows Arch. f. path. Anat.* **63**:447, 1875.

41. Schmorr, W.: *Arch. f. Gynäk.* **65**:504, 1902.

42. Landsteiner, K., and Wiener, A. S.: *Proc. Soc. Exper. Biol. & Med.* **43**:223, 1940.

Rh factor in intergroup hemolytic transfusion reactions (Wiener, Sonn and Hurst⁴³) plus the observation that women who had such reactions often had stillbirths or erythroblastotic infants (Levine and his associates⁴⁴) suggested that the Rh factor was the antigen in erythroblastosis fetalis. According to Levine and his associates, an "Rh negative mother" bearing an "Rh positive fetus" may become sensitized to the Rh factor. The Rh agglutinins thus produced pass back through the placenta, into the fetus, causing destruction of the blood of the fetus and giving rise to one or another manifestation of erythroblastosis. About 10 per cent of marriages involve a wife whose erythrocytes do not contain the Rh factor and a husband whose erythrocytes do contain it.

The clinical picture in erythroblastosis fetalis, or kernicterus, can be characterized as occurring primarily in infants that are apparently normal at birth, the labor being uncomplicated. The jaundice usually appears before the second day and is intense. Shortly after the appearance of the jaundice, there occurs evidence of involvement of the central nervous system, followed by a progressive illness, and death in a great many cases on or before the fifth day of life. Usually, one finds that more than one member of the family has had a similar type of involvement. The jaundice generally appears early, even a few hours after birth, but may be delayed as long as a number of days. In an occasional case the jaundice may not make its appearance until the infant is several weeks old. The jaundice increases rapidly and soon becomes intense. Symptoms suggestive of nervous system involvement are generally prominent and appear shortly after the onset of the jaundice, although in a few cases they may be delayed for two or three days or even weeks. These symptoms are chiefly spasticity of the extremities, opisthotonos, strabismus, spasms of the muscle, epileptiform attacks and, less commonly, a tendency toward drowsiness, apathy or even coma. Athetosis appears within a few weeks. The course is usually afebrile. The blood shows characteristic changes, anemia is present, and many erythroblasts are present. There is definite leukocytosis with reduction in the number of platelets. Death may occur within a few days or weeks.

In cases in which the disease is of a milder type, the infants may survive. These children invariably show evidence of some residual damage of the nervous system, which may make its appearance after a period of several weeks, months or even years. Development, both mental and physical, is markedly delayed. Early there is often difficulty

43. Wiener, A. S.; Sonn, E. B., and Hurst, J. G.: Studies on Individual Differences in Human Blood and Their Practical Applications: Pathogenesis of Congenital Hemolytic Disease (Erythroblastosis Fetalis); III. Illustrative Case Histories of A-B Sensitization, paper no. 1, Brooklyn, New York, Weiner Laboratories, 1946.

44. Levine, P.; Katzin, E. M., and Burnham, L.: *J. A. M. A.* **116**:825, 1941.

in feeding, with little gain in weight. Movements become clumsy; there is no effort to speak and there is failure to sit up or even to hold the head erect. Walking and talking are retarded or never acquired. The gait frequently is spastic and unsteady. These children will often show marked incoordination in the use of the musculature (Paul,⁴⁵ Spieler,⁴⁶ Greenwald,⁴⁷ Guthrie⁴⁸ and Hart⁴⁹). In most cases there are symptoms referable to the extrapyramidal system, namely, athetosis, tremor, choreiform movements and rigidity (Spieler⁴⁶ and Paul⁴⁵). When spasticity develops, it is of varying distribution and may be present with hyperactive reflexes and toe signs. Subsequently in many children generalized convulsions develop (Hoffmann and Hausmann⁵⁰). These convulsions may be of a focal or a generalized nature and may vary in frequency and severity. Almost all these patients will ultimately show some mental defect, such as emotional instability and intellectual retardation.

The autopsy findings are characteristic. In the acute form there is a distinct jaundice which can be found within the thalamus, the subthalamus, the lenticular nucleus, the caudate nucleus, the paraventricular gray matter, the hypothalamus and the dentate nucleus of the cerebellum. The jaundice is usually remarkably symmetric and is sharply localized to the gray matter. Within these jaundiced areas there is usually severe nerve cell damage which consists of chromatolysis, fragmentation and complete devastation of certain of the neurons (Schmorl,⁵¹ Esch,⁵² Beneke,⁵³ Hart⁴⁹ and Hoffmann and Hausmann⁵⁰). Pigment is situated both within the injured cells and free in the interstitial tissues. Generally, the cerebral cortex has a normally preserved structure, but the individual cortical neurons often show both acute and chronic changes. Pigment is not found in the cortical elements. There is often a mild proliferation of glia. A few mononuclears may be seen within the leptomeninges. In the more chronic form of the disease the histopathologic changes are similar to those described in the acute form with the exception that the pigmentation often is not seen. In such cases the most severe damage again is found within the basal ganglions and in scattered areas throughout the cerebral cortex.

The cause of the pathologic changes in erythroblastosis has for a long time remained unknown. Early it was felt that kernicterus was a

45. Paul, S.: *Arch. f. Kinderh.* **74**:38, 1924.

46. Spieler, W. G.: *Am. J. M. Sc.* **149**:345, 1915.

47. Greenwald, H. M., and Messer, W.: *Am. J. M. Sc.* **174**:793, 1927.

48. Guthrie, L. G.: *Proc. Royal. Soc. Med* **7**:86, 1914.

49. Hart, C.: *Berl. klin. Wchnschr.* **54**:71, 1917.

50. Hoffmann, W., and Hausmann, M.: *Monatschr. f. Kinderh.* **33**:193, 1926.

51. Schmorl, W.: *Verhandl. d. deutsch. path. Gesellsch.* **6**:109, 1904.

52. Esch: *Zentralbl. f. Gynäk.* **32**:976, 1908.

53. Beneke: *München. med. Wchnschr.* **54**:2023, 1907.

primary necrosis of the brain, which subsequently became pigmented. Schmorl⁵¹ expressed the belief that the necrosis within the basal structures was due to some toxin, perhaps bile or bile thrombi. Hart⁴⁹ also was of the opinion that poisoning or some other type of injury produced gradual degeneration of the ganglion cells, which then became impregnated with the bile pigment. Hoffmann and Hausmann⁵⁰ expressed the belief that the hepatitis caused liberation of some lipolytic substance which was responsible for the cerebral necrosis. They felt that there was a definite relationship between the severe disturbance of the liver and the pigmentation and necrosis of the ganglion cells in the basal nuclei, particularly since these cerebral changes occur only in cases in which definite necrosis of liver cells is observed. Wiener and Brody⁵⁴ in a very recent publication proposed the theory that the cerebral lesions in erythroblastosis fetalis result from the formation of agglutination thrombi in the vessels of the brain with simultaneous damage of the liver. They felt that the neurologic picture depended on the extent and the distribution of the original agglutination thrombi in the brain. The one difficulty with this theory is the specific localization of the lesions, in most cases, to the region of the basal nuclei. This specific localization of the lesions also suggests that there is an interrelationship between kernicterus and Wilson's disease. These two conditions have some similarity in view of the localization of the cerebral changes and their frequent association with hepatic lesions. However, Wilson's disease almost always occurs in adult life and is seldom associated with any jaundice or pigmentation of the cerebral tissues. Wiener and Brody⁵⁴ performed blood tests in cases of Wilson's disease and did not find any evidence of Rh sensitization.

A few of our cases of erythroblastosis fetalis will help illustrate some of the clinical and pathologic features of this illness.

CASE 2.—A 5 month old boy was admitted to the hospital two days before his death because of severe jaundice that had been present since birth. Tests of the mother's, the infant's and the father's blood for an Rh factor revealed that the mother's blood contained none but that an Rh factor was present in the blood of the father and the child. The infant had marked respiratory distress with crowing respirations. According to the history, on the fourth day of life he had several convulsions and since then had shown signs of spasticity. He had been nourished on subcutaneous fluid since birth and had gained little weight. The physical examination revealed an extremely thin, small child lying in opisthotonos, with the extremities appearing mildly spastic. The respirations were noisy, and there was mild dyspnea. The clinical impression at the time of admission was erythroblastosis fetalis with possible cerebral damage. A pneumoencephalogram was made shortly after his admission. It showed cerebral atrophy and questionable external hydrocephalus. The child's temperature continued to rise, and he died a few days after admission to the hospital.

54. Wiener, A. S., and Brody, M.: *Am. J. Ment. Deficiency* **51**:1, 1946.

An autopsy examination of the brain revealed prominent perivascular congestion. Coronal sections of the right cerebral hemisphere revealed some congestion but showed normal structure and normal basal nuclei.

Microscopic sections through the brain showed moderate vascular congestion with a few scattered perivascular hemorrhages. The most striking changes were limited to the nuclear structures of the basal ganglions and the cortex. Many of the cortical neurons, particularly in the inner cortical layers, showed a most severe disruption of the normal cell arrangement. The nerve cells were chromatolytic and often fragmented. Most of the cells stained poorly. In the basal ganglions only the large cells of the globus pallidus showed severe changes. Most of the cells were fragmented and were surrounded by large clear areas in this region. Often only a small fragment of the cell body still remained attached to a fairly intact nucleus. Some of the neurons showed more acute changes and were swollen and chromatolytic. The cells of the putamen were less severely involved. There was an occasional perivascular area of myelin swelling and early demyelination, particularly in the cortical and subcortical areas. Scattered areas of glial increase and even an occasional glial nodule were observed.

CASE 3.—A 6 day old white girl began to have a yellow tinge to the skin on the third postnatal day and was given 25 cc. of whole blood intramuscularly. From that time on the progress was gradually downhill. She died on the sixth day.

Autopsy revealed a fairly well nourished, full term infant. The right and left pleural cavities each contained 25 cc. of yellow fluid. Gross examination of the brain revealed slight congestion and mild greenish pigmentation. Coronal sections showed mild diffuse discoloration with definite pigmentation of the basal nuclei, most marked in the globus pallidus and the putamen. There was similar pigmentation along the hippocampal gyrus, the tegmentum of the brain stem, the olives and the dentate nucleus.

Histologic section through the brain showed marked congestion of the cerebral cortex with little other change. The neurons and the myelin seemed to be uninvolved. The most severe alterations occurred within the globus pallidus, where there were definite cell changes. These consisted chiefly of severe swelling and chromatolysis. Many of the cells had lost their staining properties and appeared as ghost cells. There was slight fragmentation of the cell processes as well as of the cell body.

Histologic section of the liver showed severe atrophy and destruction of the liver cords. There was a large number of nucleated red cells filling the liver sinuses.

EXPERIMENTAL STUDIES

The diseases covered thus far offer little information as to the possible causes of the cerebral lesions. In all these illnesses there was damage of both liver and brain. There is a possibility that these combined involvements may be coincidental and that the damage of the liver is not related to the cerebral changes. There are numerous experimental studies that offer some information regarding the possible interrelationship of hepatic and cerebral disease. As early as 1893, Hahn and his associates⁵⁵ produced nervous symptoms in dogs by the use of Eck's fistula and a meat

55. Hahn, M.; Massen, O.; Nencki, M., and Pawlow, J.: Arch. f. exper. Path. u. Pharmacol. 32:161, 1893.

diet (the Eck fistula served to eliminate the liver metabolism by connecting the portal vein with the vena cava and thus allowing the blood carrying chemical products from the intestine to pass directly into the main blood circulation and reach the nervous system).

Fuchs⁵⁶ in 1921, using the same method, also produced cerebral involvement in dogs, manifested as ataxia, tremors, twitchings and coma. Histologically, these animals showed evidence of a cellular reaction which was interpreted as encephalitis. Fuchs then fed cats guanidine hydrochloride and produced similar cerebral symptoms. He felt that the Eck fistula allowed the strongly toxic guanidine to reach the circulation, evading the liver. In England similar studies were done by Findlay.⁵⁷ Silberstein⁵⁸ in 1924, by using Eck's fistula, also succeeded in producing in dogs an encephalitis resembling epidemic encephalitis. He was able to transmit the cerebral involvement to dogs or rabbits by subdural and corneal inoculation of the filtrate of emulsified brain. In these animals there was a question of some latent virus being actuated by the process.

In 1932 Baló and Korpássy⁵⁹ produced marked cerebral involvement in 6 of 8 dogs by restricting them to a one-sided meat diet after Eck's fistula had been established. These dogs showed apathy, uncertain gait, hypotonia, amaurosis, catalepsy, convulsions, fibrillary twitchings and finally coma. The authors were unable to transmit the encephalitis to other dogs and therefore felt that there was no evidence that the encephalitis of dogs with Eck's fistula was caused by a living micro-organism. They regarded the encephalitis as an intoxication due to the exclusion of the liver. If the intoxicated dogs do not eat, the toxic symptoms disappear only to reappear after the dog begins to eat. In fatal cases there are mild changes localized chiefly to the caudate nucleus.

Crandall and Weil⁶⁰ in 1933 produced hepatic changes in dogs by ligation of the common bile duct or the pancreatic duct or by making Eck's fistula. They then obtained serum from these animals and incubated it with rats' spinal cord for twenty hours at 37 C. Beginning at the fourth day, the dogs' serum contained substances which were destructive to the rats' spinal cord in vitro. These substances were not lipases. The brains of the dogs showed spongy necrosis of the ventricular wall, diffuse nerve cell damage, demyelination and glial proliferation. These investigators felt that the cerebral damage was produced by a toxin. Finally de Jong⁶¹ in an extensive publication on catatonia did much to further

56. Fuchs, A.: *Wien. med. Wchnschr.* **71**:709, 1921.

57. Findlay, A. M.: *Brit. J. Exper. Path.* **5**:92, 1924.

58. Silberstein, F.: *Wien. klin. Wchnschr.* **37**:30, 1924.

59. Baló, J., and Korpássy, B.: *Arch. Path.* **13**:80, 1932.

60. Crandall, L. A., and Weil, A.: *Arch. Neurol. & Psychiat.* **29**:1066, 1933.

61. de Jong, H. H.: *Experimental Catatonia*, Baltimore, William & Wilkins Company, 1945, p. 132.

present knowledge as to the possible relationship of hepatic and cerebral damage. The author ligated the hepatic artery in 24 cats and noticed cerebral symptoms only in the 16 showing damage of the liver. Ligation of the renal and splenic arteries with resulting renal and splenic necrosis produced no cerebral symptoms. Dogs with Eck's fistula presented definite catatonia. De Jong, from his studies, felt that the cerebral damage is the result of a toxin liberated by the intestine and normally removed by the liver. In the liver damage and Eck's fistula such toxins are not removed and hence damage the brain. He did not feel that the process was due directly to poisoning from destruction of liver tissue itself.

HEPATIC DISEASE IN MAN

If cerebral damage actually results from the failure of the liver to detoxify the blood, then damage of the brain should be seen in some of the many persons suffering from severe hepatic disease. Little literature is available concerning such findings. Waggoner and Malamud³⁷ reported 5 cases in which ordinary hepatic disorder was associated with scattered proliferative changes of the astrocytes. In view of the paucity of material on this subject it was felt of value to study the central nervous system in 18 cases of acute, subacute and chronic disease of the liver. In all these cases the clinical history revealed only terminal evidence that the nervous system was involved, and this evidence consisted only of terminal lethargy or coma. In 8 of the cases the nervous system showed extensive changes. The alterations involved both the gray and the white matter and were at times limited to the nerve cells and at other times to the myelin elements. The demyelination was predominantly perivascular and involved both gray and white matter. In some cases it was so severe as to produce large demyelinated plaques that eradicated all underlying tissue elements. Fat granule cells were not present. None of the injured areas showed any cellular reaction. The neurons were irregularly damaged. Scattered nerve cells showed irregular chromatolysis, vacuolation and even fragmentation. Many failed to stain and appeared as ghost cells. Within the basal nuclei the most severe damage occurred in the putamen and the globus pallidus. The brain stem showed little alteration.

A few illustrative cases may serve to demonstrate the variation in cerebral involvement in cases of hepatic disease.

CASE 4.—This patient was a 6½ week old girl. Three weeks before admission the mother noticed that the baby was becoming jaundiced. On admission the infant was well developed and well nourished, and markedly jaundiced. The liver was palpable about 3 cm. below the costal margin in the midclavicular line. The spleen was also palpable. The blood on admission showed a hemoglobin content of 12.8 Gm.; the leukocyte count was 16,300. The bilirubin content of the serum in one minute was 6.6 mg., with a total of 10.5 mg., per hundred cubic centimeters. Thymol turbidity was 1 unit. The cephalin-cholesterol

flocculation test (Hanger's test), was negative at twenty-four and forty-eight hours. The blood cholesterol was 252 mg. per hundred cubic centimeters. Quantitative determination of urobilinogen in the urine revealed none, and less than 3 Ehrlich units per hundred grams was found in the stool.

Two weeks after the patient was admitted to the hospital, severe diarrhea suddenly developed with an elevation of temperature (102.8 F.). The hemoglobin content of the blood was found to be 8.9 Gm. per hundred cubic centimeters, with a red cell count of 2,350,000. An exploratory laparotomy was performed. The common duct was not found, but when methylene blue was injected into the gallbladder apparently some methylene blue was caused to go into the intestines through the common duct. The patient was discharged three weeks after admission.

A week before her last admission there developed a gradual protuberance of the abdomen. She became more irritable and began to refuse feedings. She appeared to be in definite discomfort. She scratched at accessible areas of the skin constantly. Respirations were labored. There was marked generalized icterus which had a deep green-bronze tint and involved the scleras and the mucous membranes. Many spider telangiectases were present about the scar of the previous operation in the right upper quadrant of the abdomen. Other spider nevi were also found. The liver was palpable as a firm, stony-hard mass extending 8 cm. down to the right midclavicular line. Shortly after this admission the patient's temperature began to rise; she became lethargic. She had voluminous hemorrhages by rectum, the respirations became progressively more shallow, and she died two days after admission.

Autopsy revealed a seriously malnourished, jaundiced child. The liver weighed 360 Gm. and was green, hard and finely nodular. Scattered throughout the liver, but particularly along the major portal vessels, were nodular areas that were dark green and quite soft. Histologic sections showed marked cirrhosis of the portal type. There was proliferation of the bile ducts, each of which was filled with a plug of inspissated bile. The hepatic ducts could not be found, and it was suspected that there was a congenital atresia of the hepatic ducts.

External examination of the brain showed scattered areas of light brown pigmentation over the cerebral hemispheres. Histologic sections revealed severe involvement of the entire central nervous system. The most striking alteration consisted of scattered areas of perivascular demyelination involving primarily the white matter and the inner cortical layers. This condition was most severe in the anterior parts of the cerebral hemispheres, primarily in the temporal, parietal and frontal areas. In many of these areas demyelination was fairly complete, producing a vacuolated or cystic appearance. Even in the areas of more diffuse destruction of myelin, one could detect a perivascular arrangement of the tissue injury.

The cortical neurons, particularly those in the inner two cortical layers, showed a variable degree of alteration. Many of the cells were shrunken, others were vacuolated, and still others showed definite fragmentation of the cell and body processes. The basal nuclei showed changes similar to those of the cerebral hemispheres. In these nuclei the demyelination was both focal and diffuse. The areas of more diffuse tissue injury were large and showed signs of early breakdown and vacuolation of tissue. The nerve cells throughout both the thalamus and the globus pallidus were severely injured. They showed diffuse chromatolysis and beginning fragmentation of the cell bodies. The medulla appeared unaltered. In the cerebellum there was a fairly extensive demyelination of the white matter. The granular layer of the cerebellum was narrowed, with

breakdown and vacuolation of tissue. The nerve cells throughout both the thalamus and the globus pallidus were severely injured. They showed diffuse chromatolysis and beginning fragmentation of the cell bodies. The medulla appeared unaltered. In the cerebellum there was a fairly extensive demyelination of the white matter. The granular layer of the cerebellum was narrowed, with many cells being absent. The Purkinje cells were reduced in number, and many showed marked alteration with fragmentation of the cell processes and marked irregularity of the cell outline.

Comment: In this case the patient suffered progressive cirrhosis of the liver secondary to atresia of the hepatic duct. Autopsy studies showed severe, widespread damage of the entire brain with no tendency to localize in any particular region. The changes seen in the brain were extremely similar to those described by me in a case of hemochromatosis in which cirrhosis also occurred. It certainly would appear that severe chronic damage of the liver can in many cases result in widespread injury of the brain.

CASE 5.—A 53 year old woman gave a six months' history of jaundice and loss of weight. She was obese and deeply jaundiced. The liver was palpable but not tender. Laboratory studies revealed a hemoglobin content of 9.8 Gm. per hundred cubic centimeters, with 3,471,000 red cells. The prothrombin time was 26.2 seconds against a control of 14.6 seconds. Blood urea nitrogen was 14 mg. per hundred cubic centimeters. Serum bilirubin was 16.3 mg. in one minute, with a total of 29.5 mg., per hundred cubic centimeters; these levels gradually rose to 26.7 mg. and 44.3 mg. before death. Thymol turbidity was 10 units, cephalin-cholesterol flocculation was 4 plus, and urinary urobilinogen was 45.8 mg per day.

In spite of therapy her course was downhill. Her jaundice deepened, and she became drowsy. She finally passed into a deep coma and died within ten days after admission.

At autopsy the liver weighed 1,320 Gm. It was nodular and yellowish. On cut section little recognizable tissue was present between the nodules. Histologic sections showed moderate portal fibrosis. In some areas hepatic cells were swollen and the cords disrupted. Frank necrosis was seen in a few places.

The entire brain showed most extensive involvement. Large areas of both gray and white matter were completely destroyed, leaving large demyelinated plaques. The tissue injury had a perivascular arrangement even in the more extensive areas of diffuse destruction of myelin. In those regions where the injury was less severe, there had occurred rather typical perivascular demyelination of varying degree and extent. There was no evidence of any inflammatory reaction. The vessels, though surrounded by altered tissues, revealed no changes even with special stains.

The cortex showed a similar but less severe perivascular involvement. Practically every vessel was surrounded by an area of myelin loss. The neurons were less severely damaged. Scattered nerve cells showed irregular chromatolysis. Many stained poorly and appeared as ghost cells. Generally the cell nuclei were uninvolved.

The basal nuclei also had undergone uniform and extensive perivascular demyelination. The nerve cell damage was most severe within the putamen and

the globus pallidus. The nerve cells within the thalamus were surprisingly intact in spite of an extensive perivascular demyelination. Sections through the brain stem showed little tissue change.

Comment: In this case the patient suffered from subacute atrophy of the liver. The lesions of the brain were actually identical with, but more severe than, those in the previous case in which the illness was chronic. The involvement of the brain was so severe that in many areas the demyelination resembled that seen in multiple sclerosis. The predominant perivascular nature of the lesions certainly suggests that the cerebral damage is secondary to the presence of some toxin which reaches the brain through the vascular system.

GENERAL COMMENT

In evaluating the significance of the cerebral lesions observed in cases of hepatic disease, one must take into account the actual cause of the hepatic involvement. Within recent years there has been an increasing tendency to relate many of the subacute and chronic involvements of the liver to an original infectious or epidemic hepatitis (Lucké,⁶² Wood,⁶³ Turner⁶⁴ and Cockayne⁶⁵). Since at present it is accepted that infectious hepatitis is caused by a virus which can be transmitted by the oral route (Havens,⁶⁶ Neefe and co-workers⁶⁷ and Findlay and Martin⁶⁸), the question arises whether the cerebral alterations in cases of hepatic disease are perhaps the direct result of the brain's being invaded by the causative infective agent. It is known that in patients with severe hepatic disease definite cerebral symptoms do occur. Such patients occasionally become lethargic and even comatose. Extensive focal symptoms may appear, such as muscular rigidity, hemiplegia, hemianopsia and parkinsonian syndromes (Lucké⁶²). However, in the fatal cases of infectious hepatitis, the cerebral changes, as would be expected, resemble those of an acute virus disease. The brain shows definite lymphocytic infiltration around the vessels in the meninges, the brain stem, the paraventricular system and the basal nuclei. The ganglion cells are regularly involved and show severe damage with complete destruction. Diffuse petechiae are common, and in the occasional case large hemorrhages may cause extensive injury of tissue.

62. Lucké, B.: *Am. J. Path.* **22**:471, 1944.

63. Wood, D. A.: *Arch. Path.* **41**:345, 1946.

64. Turner, R. H.; Snively, J. R.; Grossman, E. B.; Buchanan, R. N., and Foster, S. O.: *Ann. Int. Med.* **20**:193, 1944.

65. Cockayne, E. A.: *Quart. J. Med.* **6**:1, 1912-1913.

66. Havens, W. P., Jr.: *J. A. M. A.* **126**:782, 1944.

67. Neefe, J. R.; Stokes, J., Jr.; Reinhold, J. G., and Lukens, F. D. W.: *J. Clin. Investigation* **23**:836, 1944.

68. Findlay, G. M., and Martin, N. H.: *Lancet* **1**:678, 1943.

These lesions obviously do not resemble those seen in our cases, and one is forced to look elsewhere for a clarification of the pathologic observations. By far the most information is obtained from a review of the experimental studies, which seem to indicate definitely that the cerebral lesions are the result of the damage to the liver. When the liver is sufficiently damaged, it apparently allows the various intestinal toxins to reach the systemic circulation and thus to injure the brain. The exact nature of this endogenous toxin is at present unknown, but experimentally it appears to be related in some way to a high protein intake. Since this toxin is disseminated through the blood stream, one would expect the cerebral lesions to be widely disseminated throughout the brain and to have a perivascular arrangement. This is exactly what was observed in our cases and certainly lends evidence to this concept. Just why such cerebral damage is not found in every case of severe disease of the liver is not known. Certainly, the incidence of positive findings will increase with more careful study.

SUMMARY

Ever since Gower's first description of chorea associated with cirrhosis of the liver, in 1888, it has been apparent that severe disease of the liver may be accompanied by widespread cerebral involvement.

Wilson's disease comprises the best known of these hepatocerebral involvements. Many investigators feel that the hepatic damage is the most important component of this illness and may prove fatal for the patient before severe cerebral symptoms appear. Even Wilson felt that this disease was acquired and probably toxic in nature.

Hemochromatosis, which is characterized by pigmentation of the skin, diabetes and cirrhosis of the liver may also result in widespread cerebral lesions resembling those seen in Wilson's disease.

Experimental studies show that when the liver is damaged by ligation of the hepatic artery or is by-passed by means of an Eck fistula, severe cerebral damage results. These studies suggest that the lesions of the brain are the result of the action of a toxin liberated by the intestine and normally removed by the liver.

In our series of 18 cases of acute, subacute and chronic disease of the liver, cerebral damage was observed in 8 cases. The alterations involved both the gray and the white matter and were predominantly perivascular in nature. The widespread and perivascular distribution of the lesions again suggests that hepatic damage, if severe, apparently allows some toxin to reach the systemic circulation and thus to injure the brain. The nature of this endogenous toxin is at present unknown.

Notes and News

Appointments.—W. C. Thomas, assistant professor of pathology in Wake Forest College, Winston-Salem, N. C., has been appointed associate professor of pathology in Temple University, Philadelphia.

Death.—Edward R. Stitt, Rear Admiral, United States Navy, retired, died Nov. 13, 1948, aged 81. He was commissioned assistant surgeon in the medical corps of the Navy in 1889. He was a great teacher of bacteriology and of tropical diseases. The tenth edition of his "Practical Bacteriology, Hematology and Parasitology" appeared recently. His "Diagnostics and Treatment of Tropical Medicine" is a well known authority in its field. In 1945 he was awarded the Richard Pearson Strong Medal for outstanding service in tropical medicine.

Awards.—The second Francis Amory Prize of the American Academy of Arts and Sciences, a septennial award of \$21,000 for outstanding work in the alleviation or cure of urologic disorders, first made in 1940, will be equally divided among the following: Charles B. Huggins, of the University of Chicago; S. A. Waksman, of the New Jersey State Agricultural Experiment Station; G. N. Papanicolaou, of the Cornell University Medical College; A. B. Gutman, Presbyterian Hospital, New York; W. J. Koff, Holland, and G. F. Marian, Scotland.

George N. Papanicolaou, professor of clinical anatomy in the Cornell University Medical College, New York, has received the Borden Prize of the Association of American Medical Colleges for his work on the detection of cancer by cytologic examination. The Borden Prize is awarded for outstanding work in medical science by a faculty member of an American medical school.

The Ward Burdick Award of the American Society of Clinical Pathologists was given to P. R. Cannon, Chicago, for his contributions in the field of pathology.

Society News.—At the twenty-seventh annual meeting of the American Society of Clinical Pathologists in Chicago, Oct. 12 to 15, 1948, O. A. Brines, Detroit, was inducted as president, J. B. McNaught, Denver, was made president-elect and C. H. Manlove, Portland, Ore., was elected vice president. C. G. Culbertson, Indianapolis, was appointed secretary-treasurer on the resignation of A. S. Giordano, South Bend, Ind. The annual meeting of the American Association of Pathologists and Bacteriologists will be held in Boston April 15 and 16, 1949.

The Chicago Medical School Approved.—The American Medical Association and the Association of American Medical Colleges have granted full approval to the Chicago Medical School, bringing the number of medical schools in Chicago to 5 and the number in the United States to 71.

Cancer Research.—The official organ of the American Association for Cancer Research, previously published by the Ann Arbor Press, will now be published by the University of Chicago Press under the editorship of Paul E. Steiner, professor of pathology at the university.

Books Received

THE RH BLOOD GROUPS AND THEIR CLINICAL EFFECTS. By P. L. Mollison, A. E. Mourant and R. R. Race. Medical Research Council Memorandum no. 19. Pp. 74. Price 1s., 6d. London: His Majesty's Stationery Office, 1948.

The booklet is divided into three parts: the Rh groups (Race), clinical considerations (Mollison) and Rh testing (Mourant). The British classification of the Rh types with the Fisher-Race terminology is presented clearly with the help of tables and diagrams. Those who are interested in the current controversy concerning the respective merits of the Fisher-Race and the Wiener terminology will find here a well condensed presentation of one of the two points of view.

The clinician will find in Mollison's chapter a summary of practical applications to obstetric and pediatric practice. One could take exception to a statement on page 39: "... the possibility of making a presumptive diagnosis of the disease in the infant by simply testing the mother's serum for the presence of Rh antibodies has made the ability to recognize the disease from post-mortem appearances far less important than formerly." This statement is symptomatic of a tendency to neglect anatomic and microscopic studies and to depend entirely too much on serologic findings for the diagnosis of fetal erythroblastosis. There is good reason to deplore this trend. On the contrary, careful autopsies with thorough microscopic examinations are just as important now as they have ever been if physicians are to avoid the error of lumping together entirely unrelated conditions. In keeping with the preceding criticism is the cursory treatment of pathologic anatomic aspects of erythroblastosis.

The third chapter is a well written digest of Rh techniques. Those who are engaged in Rh studies will second Dr. Mourant's suggestion: "It should be appreciated by patients, doctors, and technicians alike that anyone who makes use of Rh tests is under an obligation to do everything possible to keep up supplies of both common and rare sera, lack of which is even now holding up . . . research."

The booklet can be recommended as one of the best reviews of the steadily expanding and complex field of Rh.

CORRECTION

In the June 1948 issue of the ARCHIVES OF PATHOLOGY, an obituary of Professor Bonne appeared on page 795. In it the statement was made, "Dr. Bonne spent the occupation years in Australia, returning to Java after the war."

We are now advised that the statement just quoted is incorrect and that Professor Bonne spent the occupation years in a Japanese concentration camp, where he was a great help and a source of inspiration to his fellow prisoners, several of whom have recently been seen by our informant.

SARCOIDOSIS INVOLVING THE HEART

Report of Case with Sudden Death

THOMAS M. SCOTTI, M.D.

AND

CHARLES E. McKEOWN, M.D.

RICHMOND, VA.

SARCOIDOSIS is not an uncommon disease, but death caused by it per se is infrequent. As indicated by one of the names attributed to it, "lymphogranulomatosis benigna" (Schaumann), the condition is generally benign. However, there are those who feel that this feature is overemphasized inasmuch as death of frank tuberculosis is frequently observed in patients with sarcoidosis. In patients with uncomplicated sarcoidosis there is a tendency of the lesions to regress spontaneously and constitutional symptoms are seldom produced. This is rather surprising in view of the widespread involvement of organs. When death is related directly to sarcoidosis, it occurs because the disease has interfered with the function of such vital structures as the lungs and the heart. Extensive pulmonary sarcoidosis may lead to severe respiratory embarrassment or may cause cor pulmonale (dilatation) and subsequent heart failure. Infiltration of the heart itself may produce cardiac decompensation. It is the latter feature which interests us and is the basis for this report. Recorded cases in which sarcoidosis of the heart was demonstrated at autopsy are few, and sudden death due to this condition has been noted twice in the English literature.¹ The case we present is another instance of sudden death from sarcoidosis.

REPORT OF CASE

A 26 year old Negro employed in a local chair factory, doing light manual labor, ate his usual breakfast and went to work with no complaint. Just before starting work he was sitting on a stool, singing and joking with his fellow workmen. He suddenly fell off the stool and was not seen to move thereafter. His foreman said that he could not feel the pulse of the patient, who appeared to have stopped breathing. On his arrival the ambulance surgeon found the man dead. Information obtained from the wife revealed that about eighteen months prior to the patient's death he complained of "pain around the heart." This was relieved by medicine prescribed by his physician, and there were no further pains. He was

From the Department of Pathology, Medical College of Virginia.

1. (a) Longcope, W. T., and Fisher, A. M.: J. Mt. Sinai Hosp. 8:784, 1942.
- (b) Bates, G. S., and Walsh, J. M.: Ann. Int. Med. 29:306, 1948.

rejected by the recruiting staff of the Army on two occasions: the first time because of "poor vision," and the second time for dependency. He is said to have had no disability except the "poor vision."

Autopsy (three hours after death).—The body was that of a well developed, well nourished, 26 year old Negro man. Rigor mortis and lividity were absent. No external lesion was observed. The lymph nodes were not palpable.

In the thorax, the thymus was not prominent. The pericardial sac contained about 10 cc. of clear amber fluid, and its surfaces were smooth and glistening. The pleurae were moist and smooth. There were no adhesions or fluid in the pleural cavities. The tracheobronchial lymph nodes were enlarged, measuring from 1 by 1 by 0.5 cm. to 4 by 3 by 2.5 cm. They were held together by dense fibrous tissue, but each could easily be separated from the others. On section they were grayish red or pink and of a rubbery consistency. No area of caseation or hemorrhage was observed. The nodes did not constrict any portion of the tracheobronchial tree. Nor did they infiltrate the lungs or the esophagus, although they adhered to the adjacent pleura, from which they could be readily freed. There were many similar lymph nodes next to the aorta within its arch.

The heart weighed 340 Gm. The myocardium was dark, reddish brown and firm. The right ventricle measured 0.4 cm., the left up to 1.6 cm., in thickness. The endocardium of the left atrium and the left ventricle was slightly thickened and hyalinized. The papillary muscles of the left ventricle were thick and on section presented a pale brown, homogeneous, glistening appearance. The chordae tendineae and the valves were not unusual. The circumferences of the valves were: tricuspid, 12 cm.; pulmonary, 7 cm.; mitral, 11 cm., and aortic, 7 cm. The coronary arteries showed a few small atheromatous plaques along the intima. The orifices were of normal size. The thoracic aorta disclosed a few atheromatous plaques of the intima; no evidence of syphilis was detected.

The left lung weighed 550 Gm.; the right, 650 Gm. All lobes were expanded and crepitant. The periphery of each lobe was pale and emphysematous. On section the lungs were dark grayish red. No pneumonia or edema was detected. The bronchi and the pulmonary vessels were not unusual.

The peritoneal cavity was free of fluid. The serosal surfaces were moist and smooth. The positions of the viscera were not abnormal.

The spleen weighed 225 Gm. It was firm and dark purplish red. The follicles were not clearly delineated.

The liver weighed 1,400 Gm. Its edges were sharp. The parenchyma was firm, and on section the cut surfaces were dark reddish brown. No gross lesions were observed. The gallbladder and the bile ducts were normal.

The gastrointestinal tract revealed congestion of the mucosa, especially in the stomach, and the submucosal lymph follicles of the large intestine were prominent. No other changes were observed. The pancreas was normal.

The mesenteric and paraortic lymph nodes were not remarkable. They were discrete, slightly enlarged and grayish pink on section.

The left kidney weighed 135 Gm.; the right, 150 Gm. The capsules stripped off easily, disclosing smooth red external surfaces. On section the cortices measured up to 0.7 cm. in thickness and were well delineated from the medullae. The latter were congested. The pelves, the ureters and the bladder were normal. The prostate gland and the seminal vesicles appeared normal. The testes were not examined.

The adrenal glands, the abdominal aorta, sections of lumbar vertebrae, the skull and the brain showed no lesions.

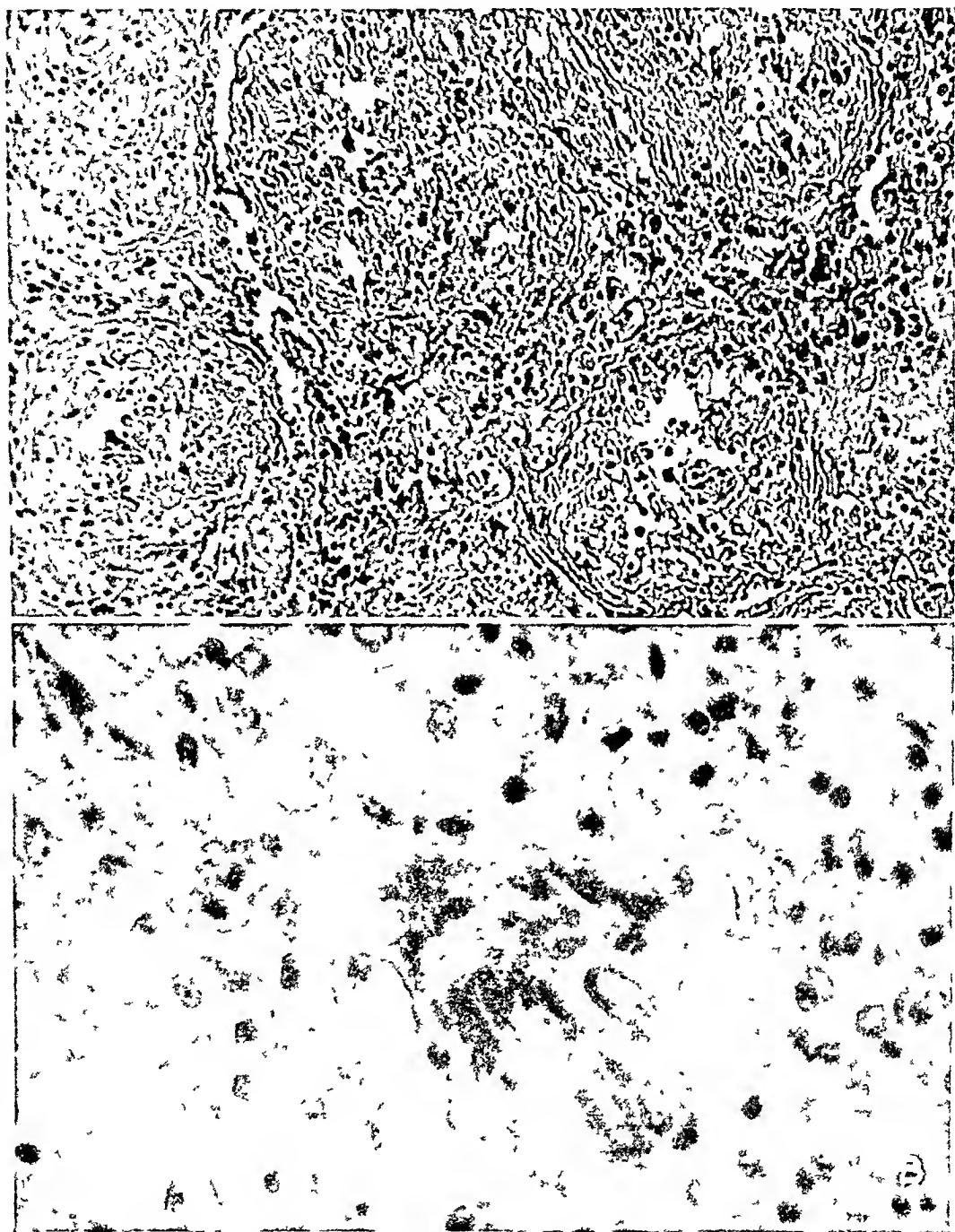


Fig. 1—*A*, section of a lymph node showing numerous, closely grouped granulomatous (sarcoid) nodules without caseation. Some of the lesions have a fibrillar background. Some hyalinization is present. Hematoxylin and eosin; $\times 100$.

B, sarcoid nodule of a lymph node showing a "Schaumann body" in the cytoplasm of a giant cell. Hematoxylin and eosin; $\times 500$.

Microscopic Examination.—Lymph Nodes: Changes were seen in the tracheobronchial and mesenteric nodes. They were more prominent in the former. Many sections showed practically the entire structure replaced by numerous closely grouped, varying-sized, round or oval nodules composed chiefly of epithelioid cells with few, many or no lymphocytes. In many instances there were a few, irregular,

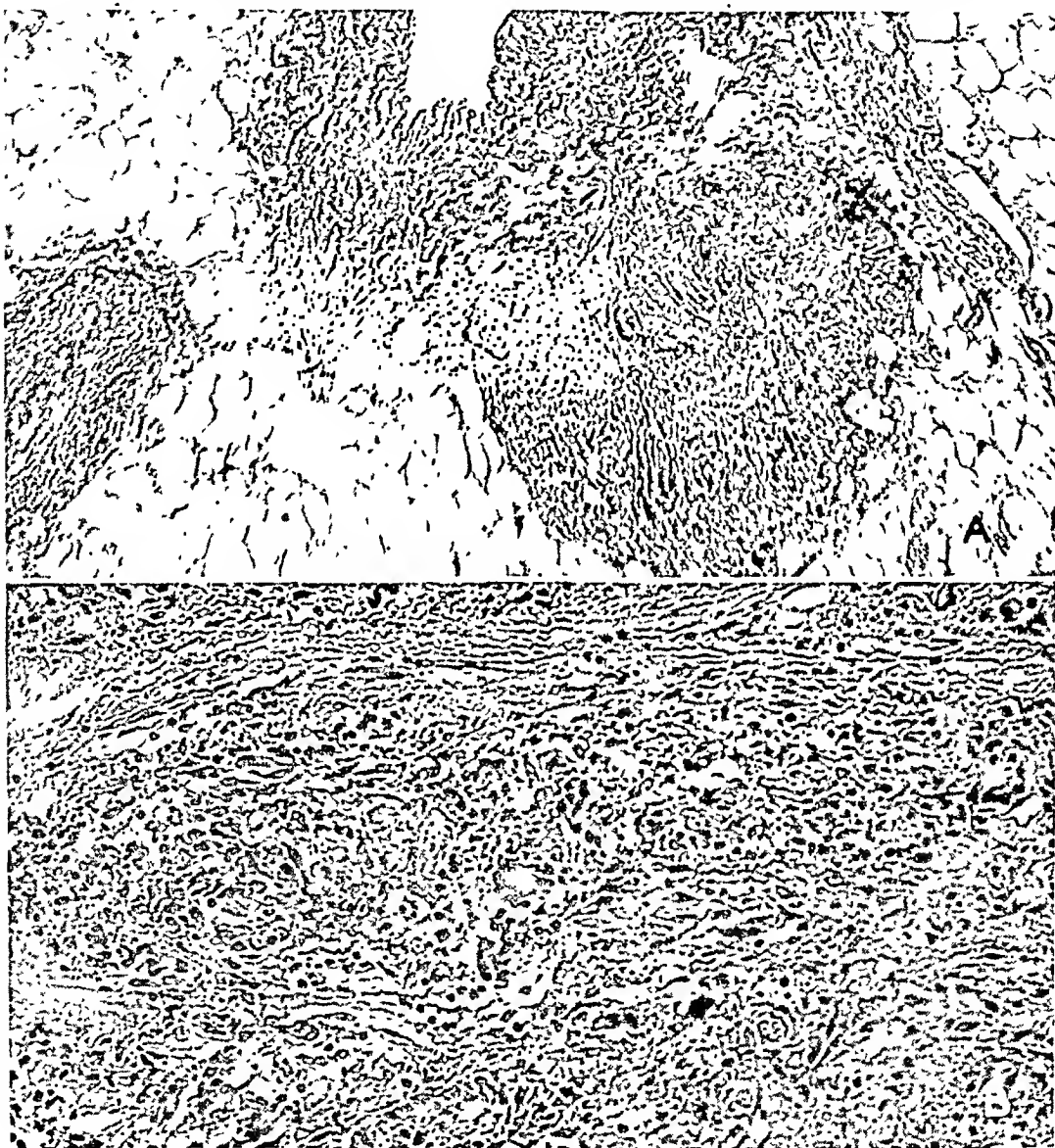


Fig. 2.—*A*, sarcoid lesion in epicardial fat adjacent to a coronary artery. Hematoxylin and eosin; $\times 75$.

B, multiple sarcoid lesions in the myocardium of the left ventricle associated with some fibrosis. Hematoxylin and eosin; $\times 100$.

multinucleated giant cells with abundant, finely granular cytoplasm and many dark-staining nuclei (8 to 40). The latter were located peripherally in some and centrally in others. Several of the giant cells contained basophilic cytoplasmic inclusion bodies made up of distorted concentric lamellas. Occasionally one of the

bodies was observed outside a giant cell. Also occasionally the cytoplasmic inclusion was an irregular, refractile, pale greenish blue, crystal-like structure. No asteroid bodies were identified. The nodules frequently had a background of argentophilic fibrils. Many nodules were completely surrounded by a thick ring of dense, hyalinized tissue which in places extended into and partially or completely replaced the lesions and in certain areas spread over large portions of the lymph node. At times concentric hyalinized rings were seen in the periphery of a lesion or about an arteriole. The hyalinized areas did not give typical reactions for amyloid with congo red and crystal violet stains, but portions stained light pink with congo red. The hyalinized tissue appeared dark green with Masson's trichrome stain for connective tissue and dark blue with Mallory's aniline blue. A striking feature of the lesions was the absence of caseation although the cytoplasm of some of the

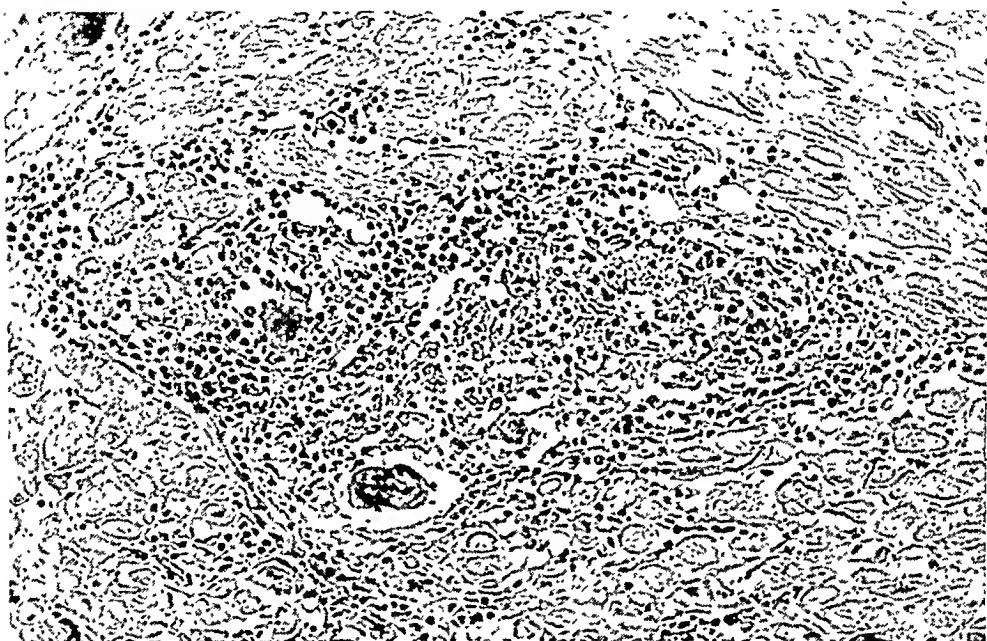


Fig. 3.—Section of a papillary muscle of the left ventricle with muscle fibers replaced to a considerable extent by the collagen associated with sarcoid lesions. More extensive fibrous replacement was noted in areas adjacent to this field. Hematoxylin and eosin; $\times 100$.

epithelioid cells was broken up. Acid-fast bacilli were not found in sections stained by the Ziehl-Neelsen technic. *Treponema pallidum* was not demonstrated by the Levaditi method.

Heart: Nodules composed of epithelioid cells, lymphocytes and occasional giant cells were found in the subepicardial fat. One of these was adjacent to a coronary artery but did not involve the wall. In sections of myocardium of the left atrium, the interventricular septum, the papillary muscles of the left ventricle and the wall of the left ventricle there were multiple granulomatous lesions like those in the subepicardial fat. They were present at all levels, including the subendocardial region. No cytoplasmic inclusion bodies were seen in the giant cells. These lesions were not as round or as ovoid in many instances as those of the lymph nodes but were nevertheless distinct. No caseation was observed in them. Fibrosis of individual lesions and of the myocardium was noted. In the interventricular septum and the left ventricle, especially in the papillary muscles of the latter, the

muscle fibers had been replaced to a considerable extent by abundant, dense, hyalinized tissue which did not give a positive reaction with the amyloid stains. Sometimes small collections of lymphocytes without the other cellular components of the lesions were seen in the myocardium. *Treponema pallidum* was not found in sections prepared by the Levaditi technic. A section of mitral valve was normal.

Lungs: Multiple distinct lesions like those described in the lymph nodes were seen throughout all sections. They lacked the dense, hyalinized tissue. No inclusion bodies were seen in the giant cells. Some of the nodules were found extending into the walls of arteries but not into the lumens. The rest of the lung tissue showed considerable hyperemia and some emphysema.

Liver: Nodules similar to those in the lungs were present. They were usually in the periphery of the lobules but were occasionally in the midzone regions. There was a moderate degree of passive congestion.

Prostate Gland: Only one section was made, and in it there was a single, tiny nodule composed of epithelioid cells and a few lymphocytes. No giant cells were noted.

Sections of other organs (aorta, trachea, esophagus, stomach, ileum, colon, spleen, pancreas, adrenal glands, kidneys, urinary bladder, thyroid gland, pituitary gland, brain and bone marrow) disclosed no granulomatous lesions. Except for the prominence of the lymphoid tissue of the stomach, the ileum and the colon, no significant changes were seen.

COMMENT

At autopsy the only remarkable finding was the pronounced thoracic and slight mesenteric lymphadenopathy, which at the time was thought to be consistent with Hodgkin's disease. The cause of the sudden death was not determined until the sections were studied microscopically. The granulomatous lesions noted in the lymph nodes, the heart, the lungs, the liver and the prostate gland were typical of sarcoidosis. Besides the characteristic cellular structure we observed in the giant cells of the lesions of the lymph node the irregular basophilic cytoplasmic inclusion bodies which were described by Schaumann² as occurring frequently in the disease. At times the inclusions were refractile and crystal-like. Asteroid bodies, which are sometimes described in sarcoidosis and which were discussed and studied histochemically by Friedman,³ were not found in our case. These stellate inclusions were first described by Wolbach,⁴ in 1911, as associated with disseminated granulomatous lesions which suggest what we now call sarcoid lesions. In the sections of lymph nodes and myocardium (that of the interventricular septum, the left ventricle and especially the papillary muscles of the latter) the extensive hyalinization accompanying the lesions, for the most part, represented collagen, the deposition of which resulted from the healing of the disease. In the sections stained with hematoxylin and eosin some

2. Schaumann, J.: *Acta med. Scandinav.* **106**:239, 1941.

3. Friedman, M.: *Am. J. Path.* **20**:621, 1944.

4. Wolbach, S. B.: *J. M. Research* **24**:243, 1911.

portions of the hyalinized areas, particularly the concentric hyaline rings of the lymph nodes, suggest the "hyalinosis" (paramyloidosis) considered by Teilum⁵ as a definite phase of the development of Boeck's sarcoid, with an allergic hyperglobulinosis of the reticuloendothelial system as the underlying primary cause. In the lymph node and heart sections which we studied, the reactions to crystal violet and congo red stains were not typical for amyloid. Only a few small areas of the lymph node stained light pink with congo red.

Careful dissection of the coronary arteries disclosed no obstructive lesions either at the orifices or along the course of the vessels to account for the myocardial fibrosis. One of the granulomatous nodules was found adjacent to a branch of the coronary artery, but it did not compress the vessel. Many sections were made to determine whether there was any obstruction of the arteries elsewhere due to sarcoid nodules, but none was detected. There was no evidence in the gross or the histologic material to indicate rheumatic fever or syphilis as a cause of the fibrosis, unless it is shown by further study that sarcoidosis is a manifestation of the latter disease as some authors⁶ contend it is in certain instances.

The sudden death is attributed to the active lesions extensively involving the myocardium, along with the prominent fibrosis. As to the mechanism of the sudden cardiac failure, we cannot say what it was. The possibilities are ventricular fibrillation or depression of the pacemaker or of the auriculoventricular conduction. As to the history of "poor vision," it suggests that the uveal tract may have been involved, as it not uncommonly is in sarcoidosis. The eyes, however, were not sectioned.

Another case of sudden death due to sarcoidosis is noted in a report in which Longcope and Fisher^{1a} recorded cases of sarcoidosis involving the heart. Six of 31 patients with sarcoidosis presented evidence during life of some derangement of heart action or showed at autopsy sarcoid lesions of the heart. Cardiac lesions were observed in 3 cases in which autopsies were made. One autopsy, made on a 40 year old Negro man, who supposedly was in good health and who dropped dead on his doorstep, disclosed large masses about the great vessels of the thorax with extensive infiltration of the pericardium and the myocardium. There was enlargement of the superficial, mediastinal, mesenteric and retroperitoneal lymph nodes. Microscopically, the condition proved to be sarcoidosis involving also the lungs, the liver, the kidneys, the spermatic cord, the cerebral dura and the skin of the penis. Another autopsy,

5. Teilum, G.: *Am. J. Path.* **24**:389, 1948.

6. (a) Bernstein, M.; Konzelmann, F. W., and Sidlick, D. M.: *Arch. Int. Med.* **44**:721, 1929. (b) Frazier, C. N., and Hu, C. K.: *Proc. Soc. Exper. Biol. & Med.* **30**:898, 1933.

on a 42 year old Negro man who had cardiac symptoms during life and who became mentally deranged and committed suicide, showed fresh and old sarcoid lesions present throughout the myocardium and the pericardium. Lesions were noted also in the pleura, the lungs, the spleen, the liver and the kidneys. There was, in addition, a dense scar in the interventricular septum. No evidence of syphilis was detected, but the authors could not exclude syphilis as a cause of the scar. In a third autopsy, on a patient who had no cardiac symptoms during life, a few lesions were found in the myocardium.

Recently Bates and Walsh¹¹ described a case of sudden death due to sarcoidosis in a report dealing with observations on 7 patients with Boeck's sarcoid. One of these, a 31 year old Negro man, complained of anorexia, weakness, and pain in the calves for two months, and a diagnosis of pseudohypertrophic muscular dystrophy was made. Later, however, the presence of sarcoidosis was established by examination of a surgically removed epitrochlear lymph node. Ten months after the onset of symptoms there was "sudden and unexpected death." At autopsy sarcoid lesions were found throughout the myocardium (both ventricles, the interventricular septum and the papillary muscles) associated with fibrosis. Lesions were also noted in the epicardium, the lungs, the liver, the kidneys, the spleen, the tracheobronchial lymph nodes and the voluntary muscles. Before death there were no symptoms suggesting cardiac involvement, although the authors felt that the persistent tachycardia (90 to 120), even when the patient was afebrile, should have been a warning that the myocardium was invaded by sarcoidosis.

As far as we have been able to determine, the article by Bernstein, Konzelmann and Sidlick^{6a} was the first in which there was reported an autopsy of a patient in whom this disease had involved any of the structures of the heart. Their patient was a 52 year old white man with no apparent cardiac complaints. He had multiple lesions of the skin and a chronic respiratory ailment. He had dyspnea due to the respiratory disease and hydrothorax and died of bronchopneumonia. Lesions which were considered by the authors to show the histologic changes of sarcoidosis were found in the skin, the bronchial mucosa and the mucosa of the ileum. Nodules were also present in the epicardium, situated along the coronary vessels. Grossly the superficial muscle fibers were invaded for a distance of 4 or 5 mm., but there was no histologic description of any lesion of the myocardium or the endocardium.

Schaumann⁷ gave an account of 4 cases of sarcoidosis in which autopsies were made. One of the patients, a 45 year old white man,

7. Schaumann, J.: *Brit. J. Dermat.* 48:399, 1936.

had extensive cutaneous and visceral involvement and died of cardiac failure. The author felt that the "asthenia of the heart, arising both from the increased resistance produced by the lung-lesion and from the localization of the disease in the heart, was presumably the cause of the death, although it seems impossible to gauge what part the advanced destruction of the haematopoietic apparatus played in the fatal issue." At autopsy he found enlargement of the heart, chiefly hypertrophy and dilatation of the right chamber. Histologically, he noted sarcoid lesions in the epicardium, and in the interstices of the myocardium there was "some slight cell infiltrate" but no "epithelioid foci." Besides the cardiac lesions and the extensive involvement of the skin and the lungs, nodules were found in the liver, the spleen, lymph nodes (cervical, axillary, inguinal, tracheal, bronchial, mesenteric, iliac and those in the hilus of the spleen and the liver), the capsules of the kidneys, the bone marrow, the tendon sheaths (hand) and the tonsils.

Nickerson⁸ reported 6 cases of sarcoidosis in which autopsies were made. In 5 of these there were no outstanding gross findings except the major disease causing death. The most constant feature he found was unexplained splenomegaly with or without abdominal lymphadenopathy. In 1 case there was an acute overwhelming sarcoidosis. The patient, a 58 year old Negro woman, had dyspnea related to the pleural effusion. The heart sounds were regular, rapid and weak. No murmurs were heard. At autopsy there were multiple nodules in the parietal pericardium and a few solitary lesions in the myocardium and subendocardial fibrous tissue. Also involved were the parietal pleura, the lungs, the spleen, the liver, lymph nodes and an eyelid.

Another case of generalized sarcoidosis with autopsy was recorded by Spencer and Warren.⁹ The patient was a 51 year old man who had no clinical evidence of cardiac involvement. His death was due to obstruction of the airway following edema of the larynx. Autopsy disclosed sarcoid lesions in the myocardium as well as in the lungs, the skin, the liver, the spleen, lymph nodes (bronchopulmonary, tracheal, paravertebral, iliac and inguinal), the trachea, the thyroid gland and the kidneys.

Extensive involvement of the myocardium with fibrosis and lesions of the endocardium, including nodules on the mitral valve, were disclosed in a case presented by Cotter.¹⁰ The patient, an 18 year old Negro youth, had clinical evidence of cardiac derangement and died of progressive myocardial failure. Autopsy revealed other lesions in

8. Nickerson, D. A.: *Arch. Path.* **24**:19, 1937.

9. Spencer, J., and Warren, S.: *Arch. Int. Med.* **62**:285, 1938.

10. Cotter, E. F.: *Arch. Int. Med.* **64**:286, 1939.

the lungs, peritracheal and hilar lymph nodes, the liver, the spleen, a testicle, the wall of the alimentary tract, subcutaneous tissue and underlying muscle.

A review of the literature dealing with sarcoidosis of the heart was published by Johnson and Jason¹¹ in 1944. These authors also reported a case of their own. The patient, a 24 year old Negro man, had symptoms referable to the cardiac lesions which caused his death. They found massive infiltration of the myocardium, not unlike that in Cotter's case, together with lesions of the epicardium, the endocardium, the visceral pleura, the lungs, the spleen, the liver, thoracic and upper abdominal lymph nodes and the testes. Johnson and Jason stated that "a review of the case reports of Boeck's sarcoid with cardiac lesions is hampered by the lack of uniformity in diagnostic criteria and the fact that many authors are of the opinion that sarcoidosis is a proliferative and non-caseating form of tuberculosis." As they pointed out, conditions have been reported as "atypical tuberculosis," "specific myocarditis," "granulomatous myocarditis" or "myocarditis of unknown cause" which may represent sarcoidosis of the heart. One report, among others, to which they refer is that of Brosig's concerning a patient who died suddenly. According to these authors, the lesions in that case which extensively involved the mediastinal nodes and the heart cannot be distinguished microscopically from sarcoidosis.

In a case of coexistent pulmonary asbestosis and sarcoidosis discussed by Skavlem and Ritterhoff,¹² sarcoid lesions were found incidentally in the myocardium of the right and left ventricles. Those in the right ventricular myocardium were accompanied by considerable fibrosis. The patient was a 42 year old white man who worked in an asbestos plant for twenty-five years. He had no cardiac symptoms at any time. Dyspnea was related to the lung disease. Autopsy disclosed, in addition to the pulmonary and myocardial involvement, sarcoid nodules of the tracheobronchial lymph nodes, the spleen, the liver, the kidneys and the diaphragm. Interestingly, the authors found both types of characteristic inclusions in the lesions, "Schaumann bodies" and asteroid bodies, a combination which they did not observe in other reported cases.

Hauser¹³ presented 19 cases of pulmonary sarcoidosis, in 4 of which autopsies were made. One of the patients, a Negro woman aged 27 years, died of respiratory and cardiac failure (cor pulmonale) following a period of progressive dyspnea. Sarcoid lesions were seen in the pericardium but nowhere else in the heart. The following organs were

11. Johnson, J. B., and Jason, R. S.: *Am. Heart J.* **27**:246, 1944.

12. Skavlem, J. H., and Ritterhoff, R. J.: *Am. J. Path.* **22**:493, 1946.

13. Hauser, H.: *J. Oklahoma M. A.* **39**:395, 1946

Sarcoidosis Involving the Heart. 13 Cases in Which Autopsies Were Made

Author and Patient	Clinical Evidence of Cardiac Involvement	Cause of Death	Lesions of Heart	Lesions of Other Organs
Bernstein and others, ^{8a} 52 yr. white man	None (dyspnea due to respiratory disease and hydrothorax)	Bronchopneumonia	Epicardium	Skin, bronchial mucosa, intestinal mucosa
Schaumann, ² 45 yr. white man	Congestive heart failure (due to cor pulmonale and possibly to cardiac lesions)	Cardiac failure (due to cor pulmonale and possibly to cardiac lesions)	Epicardium	Lungs, liver, spleen, skin, lymph nodes, tonsils, bone marrow, tendon sheaths (hand), capsule of kidneys
Niekerson, ⁸ 58 yr. Negro woman	None (dyspnea due to pleural effusion)	Acute, overwhelming sarcoïdosis	Parietal pericardium, myocardium, subendo-cardial fibrous tissue	Parietal pleura, lungs, liver, lymph nodes, spleen, eyelid
Spencer and Warren, ⁹ 51 yr. man	None	Obstruction of airway (edema of larynx)	Myocardium	Lungs, skin, liver, spleen, lymph nodes, kidneys, trachea, thyroid gland
Cotter, ¹⁰ 18 yr. Negro man.....	Progressive myocardial failure	Sarcoidosis of heart	Myocardium Endocardium	Lungs, lymph nodes, liver, spleen, alimentary tract, subcutaneous tissue and underlying muscle, testis
Longcope and Fisher, ^{1a} 40 yr. Negro man	None known	Sarcoidosis of heart—death sudden	Pericardium Myocardium	Lymph nodes, lungs, liver, kidneys, spermatic cord, cerebral dura, skin (penis)
42 yr. Negro man	Stokes Adams syndrome; auriculoventricular dislocation	Suicide	Pericardium Myocardium	Pleura, lungs, spleen, liver, kidneys
Johnson and Jason, ¹¹ 24 yr. Negro man	None	*	Myocardium	*
	Premature ventricular beats; ventricular tachycardia and severe congestive heart failure	Sarcoidosis of heart	Epicardium Myocardium Endocardium	Visceral pleura, lungs, spleen, lymph nodes, liver, testis
Skavlen and Ritterhoff, ¹² 42 yr. white man	None (dyspnea related to lesions of lungs)	Respiratory failure (co-existent asbestosis and sarcoidosis)	Myocardium	Lungs, lymph nodes, spleen, liver, kidneys, diaphragm
Hauser, ¹³ 27 yr. Negro woman....	Progressive dyspnea (respiratory and cardiac: cor pulmonale)	Respiratory and cardiac failure (cor pulmonale)	Pericardium	Lungs, bronchi, pleura, spleen, right kidney, lymph nodes
Bates and Walsh, ^{1b} 31 yr. Negro man	Persistent tachycardia	Sarcoidosis of heart—death sudden	Myocardium Epicardium	Lungs, liver, kidneys, spleen, tracheo-bronchial lymph nodes, voluntary muscles
Scott and McKown: Arch. Path., present issue, 26 yr. Negro man	No accurate history, but apparently no symptoms—said to have had "pain around heart" once	Sarcoidosis of heart—death sudden	Epicardium Myocardium	Lymph nodes, lungs, liver, prostate

* The details were not given in the article.

also involved: lungs, bronchi, pleura, spleen, right kidney and lymph nodes (mediastinal, left inferior deep cervical, pretracheal, celiac, hepatic and paraortic).

In these reported cases of sarcoidosis of the heart noted in the English literature (see accompanying table) only 3 of the 13 patients had cardiac symptoms attributable to the lesions of the heart alone. Another had no symptoms suggesting cardiac involvement, but we felt that the persistent tachycardia, which was present even when the patient was afebrile, should have been a warning that the myocardium was invaded by sarcoidosis. Five patients died as a result of myocardial involvement; 3 of these, 2 of whom were presumably in good health during life, died suddenly. Two died of cardiac failure due to increasing pulmonary resistance (*cor pulmonale*) caused by extensive infiltration of the lungs, and in one of these, according to the author, weakness of the heart was possibly due also to cardiac localization of the lesions.

SUMMARY

A case of sarcoidosis involving the heart, with sudden death, is presented. There were numerous lesions in the myocardium, associated with considerable fibrosis. Lesions were also present in the epicardium, in thoracic and mesenteric lymph nodes and in the lungs, the liver and the prostate gland.

Twelve cases of sarcoidosis of the heart reported by other authors, in which autopsies were made, are briefly reviewed. Among these were 2 cases with sudden death.

CHRONIC INFLAMMATORY LESIONS OF SKELETAL MUSCLE IN RHEUMATOID ARTHRITIS AND IN OTHER DISEASES

M. A. OGRYZLO *

TORONTO, CANADA

DESPITE the fact that voluntary muscle normally constitutes about 50 per cent of the body mass, pathologic studies of this tissue are infrequently reported. This is remarkable when one considers the profound changes which occur in the skeletal muscles in many of the more chronic systemic diseases. New interest has been stimulated in this subject by recent reports of inflammatory lesions occurring in skeletal muscle in patients with rheumatoid arthritis.

While the outstanding feature of rheumatoid arthritis is the involvement of the joints, the rapid wasting and the extreme degree of atrophy of muscles which accompany the more severe forms of the disease are of considerable interest. The latter feature is not necessarily limited to those muscles directly associated with the involved joints, but often is more generalized. Moreover, it is usually of greater severity than can be accounted for on the basis of spasm, disuse or emaciation of muscles.

Indicative of the systemic nature of the disease, Curtis and Pollard,¹ in 1940, first reported on the perivascular infiltrations of inflammatory cells occurring in skin and muscle of patients with rheumatoid arthritis. The cells were chiefly lymphocytes and were found in the skeletal muscles of 5 of 11 patients from whom specimens of muscles were taken for biopsies. This included 2 of 3 patients whose symptoms conformed to the criteria of the so-called Felty's syndrome. No conclusion was drawn other than that the lesions probably indicated the presence of some generalized infectious process.

In 1945 Freund and associates² noted small inflammatory nodules in the muscles of amputated lower limbs from a patient with rheumatoid arthritis and substantiated this finding by consistently demonstrating lesions in biopsy specimens from 14 patients who had typical rheumatoid arthritis. This work was extended by Steiner and co-workers,^{3a} who

* National Research Council Fellow in Medicine, University of Toronto.

From the Departments of Medicine and Pathology, University of Toronto, and the Medical Service, Toronto General Hospital.

1. Curtis, A. C., and Pollard, H. M.: *Ann. Int. Med.* **13**:2265, 1940.

2. Freund, H. A.; Steiner, G.; Leichtentritt, B., and Price, A. E.: *Science* **101**:202, 1945.

3. Steiner, G.; Freund, H. A.; Leichtentritt, B., and Maun M. E.: (a) *Am. J. Path.* **22**:103, 1946; (b) footnote, p. 120.

described the same lesions in all of 9 patients with rheumatoid arthritis, one of whom was encountered in a series of 196 controls. Small lymphocytic infiltrations were found in only one other person, a patient with subacute bacterial endocarditis superimposed on old rheumatic heart disease. The remainder of the controls showed no such lesions. Subsequent examination of 5 additional patients with rheumatoid arthritis showed identical lesions.^{3b}

The lesions consisted of nodular and focal collections of lymphocytes and plasma cells, together with occasional mast cells, polymorphonuclear leukocytes and eosinophils. In some of the larger nodules epithelioid cells were noted. There was no evidence of necrosis. The nodules were found chiefly in the perimysium separating muscle bundles and in the endomysium infiltrating between the individual muscle fibers. Lesions of the epimysium were uncommon. Some of the focal collections occurred around and in close relation to small blood vessels, and actual infiltration of the vessel walls was described. Associated degenerative change of the muscle fibers was the rule.

These authors regarded the lesions as specific and essential findings in rheumatoid arthritis, and they considered the associated muscle degeneration as a consequence of the inflammatory lesion. Their occurrence in muscles in cases of rheumatoid arthritis has been confirmed by subsequent investigations,⁴ although they have not been encountered in all cases. The application of this new finding as an aid to diagnosis has been advocated.⁵

OBJECT OF THE PRESENT STUDY

The present study was undertaken in order to determine the specificity or the nonspecificity of the lesions under discussion. Sections of skeletal muscle taken routinely during the past ten years in the departments of pathology, surgical pathology and neuropathology of the Toronto General Hospital were carefully reviewed, as well as additional sections obtained in a group of selected cases. The latter were chosen from among cases of diseases in which pathologic changes of muscle might be expected to occur. In all a total of 158 cases were examined. Sections were routinely stained with hematoxylin and eosin.

RHEUMATOID ARTHRITIS

Fifteen cases of chronic rheumatoid arthritis which came to autopsy were reviewed. All were cases of long standing, the disease having been present for one

4. (a) Gibson, H. J.; Kersley, G. D., and Desmarais, M. H. L.: *Ann. Rheum. Dis.* 5:131, 1946. (b) Clawson, B. J.: *Am. J. Path.* 22:647, 1946. (c) de Forest, G. K.; Bunting, H., and Kenney, W. E.: *Am. J. Med.* 2:40, 1947.

5. Steiner and others.³ de Forest and others.^{4c}

and a half to fifty-five years. In two thirds of the cases the history was in excess of five years' duration. The ages of the patients varied from 14 to 76 years at the time of death; all but 1 were over 40 years of age.

Sections were cut from stock material, the tissue customarily being taken from the rectus abdominis muscle. Cellular accumulations or infiltrations probably indicative of inflammatory lesions were found in 3 of the 15 cases. In the sections from 2 of these 3 cases there were small nodular or focal collections of cells consisting chiefly of lymphocytes, a few plasma cells and occasional eosinophils and polymorphonuclear leukocytes. The nodular collections were distinctly microscopic in size and were situated in the endomysial (fig. 1) or the perimysial (fig. 2) tissues. The endomysial lesions within the muscle bundles were sometimes associated with evident degenerative changes of one or several muscle fibers (fig. 3). In the perimysial tissue between muscle bundles the lesions were often perivascular or paravascular in location, but infiltration of the vessel walls was not noted. In both cases there was shown a mild to moderate degree of atrophy of muscle, as evidenced by shrinkage and vacuolation of single or at most a few muscle fibers, proliferation of sarcolemma nuclei and fatty replacement. In other areas similar degenerative changes were commonly seen in the muscle fibers without evidence of any inflammatory reaction. In the third case the muscle contained a diffuse type of lesion (fig. 4) accompanied by mild degenerative changes of the muscle.

The sections representing the remaining 12 cases disclosed no lesions of an inflammatory nature despite the fact that degenerative changes were noted in 3 instances. This was of equal or greater severity than in those cases in which inflammatory lesions were demonstrated.

Biopsy material from muscles of limbs was examined in 5 cases of active rheumatoid arthritis varying in duration from one to twenty-six years. Cellular lesions were encountered in 2 cases, consisting largely of small focal collections of lymphocytes. Evidence of atrophy of muscle was present in both. No lesions were found in the 3 remaining cases in spite of moderate to severe degenerative changes in the muscles.

The technic employed in these 20 cases of rheumatoid arthritis was that routinely used in examining surgical material. An average of three sections were cut in each instance. In cases showing no lesions examinations were repeated, additional blocks of muscle being used.

ANKYLOSING (STRÜMPPELL-MARIE) SPONDYLITIS

Owing to the difference of opinion which prevails in regard to the nature of this disease, the findings are not included with those of rheumatoid arthritis. Sections of muscle were examined from 6 cases: In 3 of these cases the tissue had been obtained for biopsy, and in 3 it was from autopsy material. The duration of illness varied from four to nine years. Degenerative changes of moderate degree were present in 3 of the cases, but focal inflammatory lesions of the type described in the foregoing section were not encountered. In 1 instance there were occasional lymphocytes and plasma cells about one or two small arterioles, but these were not impressive and might readily be overlooked.

OTHER TYPES OF ARTHRITIS

Biopsy material from 2 cases of gonorrheal arthritis revealed no inflammatory lesions, although a mild degree of muscle atrophy was present.



Figures 1-4

Sections were obtained for biopsy from 2 patients with gout. In one instance the tissue revealed nothing. In the other the specimen, removed from an area adjacent to tophaceous deposits, contained diffuse and focal lymphocytic infiltrations. A third patient was clinically suspected to have gout, but the history was not characteristic and no tophaceous lesions were demonstrated at autopsy to support the diagnosis; yet distinct periarteritis was revealed microscopically. The lesions of muscle consisted of perivascular infiltrations of lymphocytes and plasma cells (fig. 5), the cells often infiltrating the vessel walls. The affected arterioles were considerably thickened and the lumens narrowed. Cellular proliferation within the walls of the vessels was a prominent feature, with areas of fibrinoid change. The patient was a 57 year old man with a history of recurrent arthritis of multiple joints of eight years' duration. Treatment with cinchophen had been started one year before death but was discontinued after three months because of a complicating hepatitis. The immediate cause of death was bronchopneumonia with congestive heart failure. Viscera showed lesions similar to those found in muscle but of a milder degree.

Small focal and perivascular collections of lymphocytes were encountered in muscle from a 48 year old woman with a twelve months' history of nonspecific arthritis involving the small joints of the hands. Death was due to cerebral hemorrhage. The history had not been obtained in sufficient detail to include this case as one of rheumatoid arthritis, and the joints had not been examined at autopsy. Microscopically, there was no evidence of muscle degeneration.

Diffuse focal and perivascular lesions were also found in muscle from a 45 year old woman with a five months' history of acute polyarthritis. Death was attributed to sulfonamide intoxication. The inflammatory lesions consisted of lymphocytes, plasma cells, a high proportion of large, pale mononuclear cells and occasional polymorphonuclear leukocytes.

DERMATOMYOSITIS

Biopsies made in 4 cases of dermatomyositis, in each of which the clinical picture was characteristic, all demonstrated well marked inflammatory lesions. The most prominent were focal collections of lymphocytes, plasma cells, occasional large mononuclear cells and, rarely, a few polymorphonuclear leukocytes (fig. 6). In some areas there was extension of the infiltrations between individual muscle fibers, while in 2 cases a diffuse infiltration of cells also was present. Perivascular collections were commonly encountered both within and between the muscle bundles. In 2 cases degenerative changes in the muscle fibers were mild; in the others they were of a moderate degree.

DISSEMINATED LUPUS ERYTHEMATOSUS

Sections of muscle were examined from 4 patients with disseminated lupus erythematosus who came to autopsy. No inflammatory lesions were noted in the muscle of 1 patient, while that of the remaining patients contained obvious lesions.

Fig. 1.—Focal chronic inflammatory lesions of the endomysial tissue of skeletal muscle in a case of rheumatoid arthritis. Two small vessels are present in the upper left hand corner. $\times 160$.

Fig. 2.—A focal or nodular collection of chronic inflammatory cells in the perimysial tissue between muscle bundles in a case of rheumatoid arthritis. The muscle is well preserved. $\times 250$.

Fig. 3.—Small endomysial collections of cells in a case of rheumatoid arthritis. $\times 300$.

Fig. 4.—Diffuse infiltration of muscle in a case of rheumatoid arthritis. There is considerable edema of the tissue with wide separation of the fibers. $\times 250$.

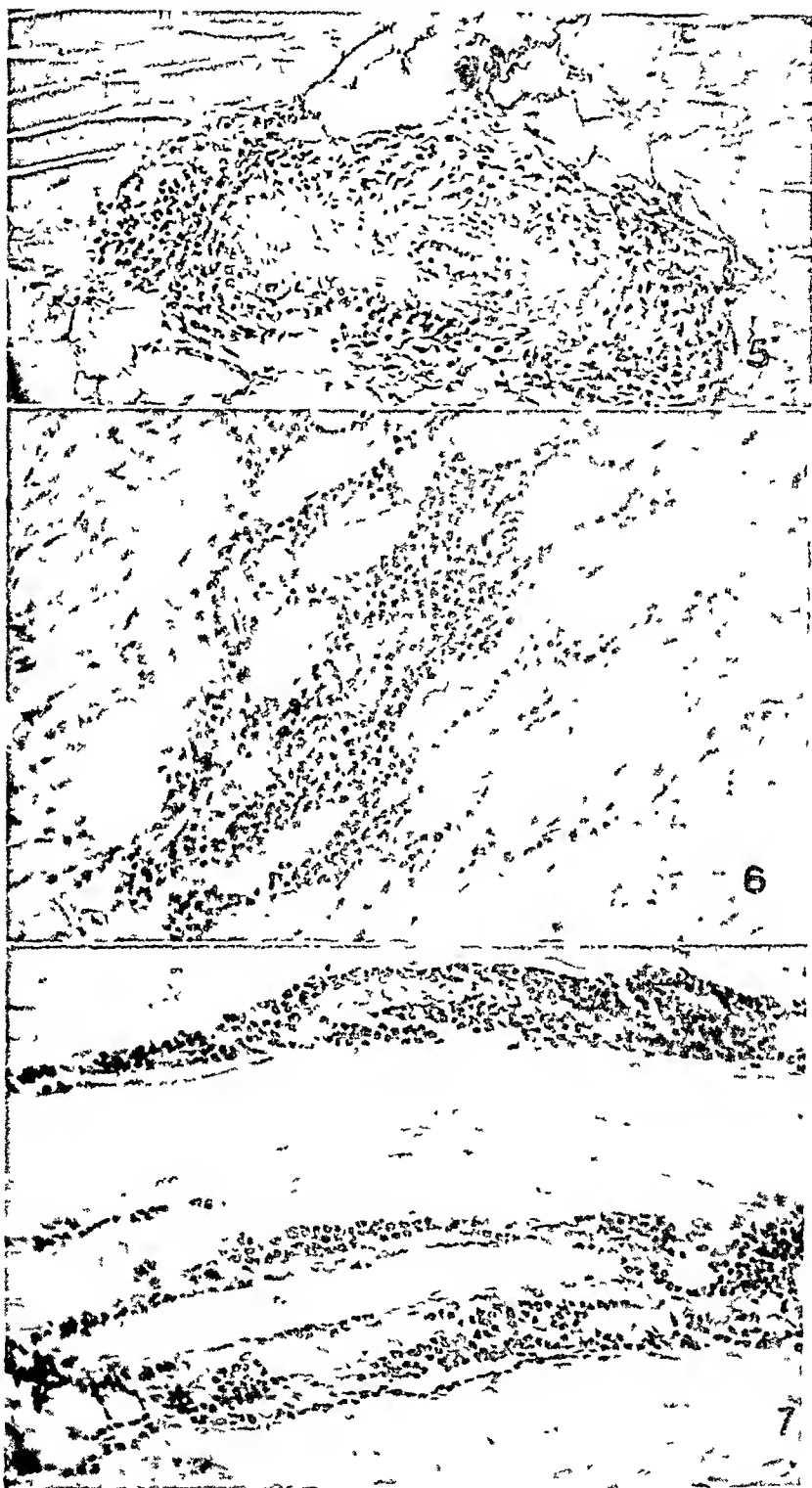


Fig. 5.—A section of muscle from a patient with questionable gout who had been treated with cinchophen nine months previously. Distinct periarteritis is present. $\times 160$.

Fig. 6.—A nodular collection of chronic inflammatory cells in the endomysial tissue of muscle from a patient with dermatomyositis. $\times 250$.

Fig. 7.—Focal collections of chronic inflammatory cells in muscle in a case of disseminated lupus erythematosus. $\times 225$.

These included focal (fig. 7) and perivascular collections of cells, with occasional diffuse infiltrations between individual muscle fibers. The cells comprised chiefly lymphocytes, occasional plasma cells and a few polymorphonuclear leukocytes or eosinophils. Degeneration of muscle fiber was mild in 2 cases and not evident in the others.

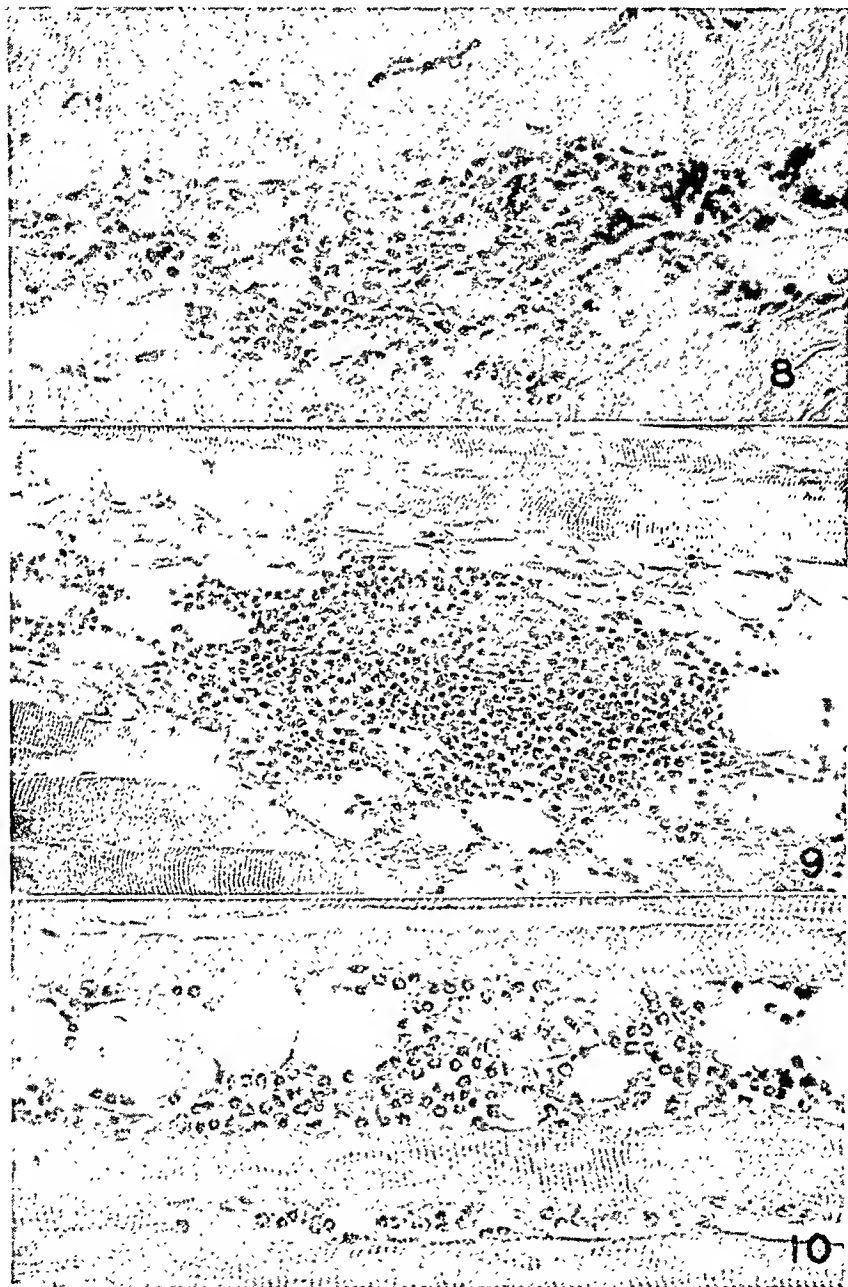


Fig. 8.—An endomysial collection of chronic inflammatory cells, not related to any vessel, in a case of periarteritis nodosa. $\times 250$.

Fig. 9.—A nodular lesion of perimysium in a case of Addison's disease. $\times 225$.

Fig. 10.—A small endomysial lesion in a case of hyperthyroidism. $\times 300$.

PERIARTERITIS NODOSA

In sections of muscle obtained from 2 patients with periarteritis nodosa, from one at biopsy and from the other at autopsy, the arterial and periarterial lesions—comparable to those illustrated in fig. 5—were the prominent feature, though small focal collections of cells were present also in the endomysium. The cells were mainly lymphocytes, odd plasma cells and a few large mononuclear cells. The specimen obtained at biopsy contained a large proportion of eosinophils. In 1 other case examined post mortem, small focal collections were the only lesions present (fig. 8); the typical periarteritis was not evident in the muscle sections.

ADDISON'S DISEASE

In 7 cases of Addison's disease muscles were examined at autopsy. In 3 of these the sections contained lesions. In 1 case there were large focal (fig. 9) and small perivascular collections of lymphocytes and plasma cells, with a few large mononuclear cells. In 2 others minute collections of lymphocytes, numbering about 15 to 20 cells, were noted, usually in relation to single degenerating muscle fibers. No lesions were observed in the remainder of the cases, though moderately severe atrophy of muscle was evident in 1.

HYPERTHYROIDISM

In 2 of 3 cases of hyperthyroidism that came to autopsy the muscles contained lesions. In one instance the lesions were focal (fig. 10), and in the other the lesion was diffuse, with considerable atrophy and edema of muscle. The cells were predominantly lymphocytes; a few plasma cells and occasional polymorphonuclear leukocytes or large mononuclear cells were seen. One section of extraocular muscle in a case of malignant exophthalmos showed considerable edema, atrophy and diffuse lymphocytic infiltration.

MYXEDEMA

In 4 cases of myxedema sections disclosed no inflammatory lesions. In 1 case multiple clusters of small round or oval cells with darkly staining nuclei were observed. These appeared to be located within the sheaths of the individual muscle fibers and resembled sarcolemma cells (fig. 11).

SUBACUTE BACTERIAL ENDOCARDITIS

Lesions of muscles were found in only 2 of 6 cases of subacute bacterial endocarditis examined post mortem. In one the lesions consisted of tiny focal collections of lymphocytes, plasma cells and occasional polymorphonuclear leukocytes, usually in relation to single degenerating muscle fibers. In the other the involved muscle contained large focal collections (fig. 12) of lymphocytes and plasma cells and, in addition, a high proportion of polymorphonuclear leukocytes and large, pale mononuclear cells. The muscle fibers showed moderately severe degenerative changes without necrosis.

RHEUMATIC FEVER

No inflammatory lesions of muscle were encountered in 5 cases of rheumatic disease of the heart with microscopic evidence of active myocarditis. In 1 case the history was of four months' duration, and there was an acute and severe reaction in the heart muscle.

MYASTHENIA GRAVIS

Biopsy material was obtained from 10 patients said to be suffering from myasthenia gravis. In 2 cases the sections of muscle revealed small focal lymphocytic collections (fig. 13). Muscle degeneration and atrophy were present in all instances, and in some were severe. The diagnosis of myasthenia gravis was not verified in all instances, however. Six of the patients were not hospitalized, and their diagnosis must remain in doubt.



Fig. 11.—Dense proliferations of sarcolemma cells in skeletal muscle in a case of myxedema. The clusters of cells occur in relation to degenerating fibers. $\times 230$.

Fig. 12.—A large nodular lesion of endomysium in a case of subacute bacterial endocarditis. Many large mononuclear cells and polymorphonuclear leukocytes are present, as well as lymphocytes and plasma cells. $\times 250$.

MUSCULAR ATROPHY AND MUSCULAR DYSTROPHY

Included in this group are 4 cases of amyotrophic lateral sclerosis, 3 cases of muscular dystrophy and 1 case each of progressive muscular atrophy, Charcot-

Marie-Tooth disease (progressive neuropathic [peroneal] muscular atrophy), juvenile myopathy and diffuse neuronal degeneration, in all of which moderate to severe muscle degeneration was observed without evidence of inflammatory lesions. In 1 case of fascioscapulohumeral atrophy the muscle contained small, focal collections of lymphocytes with occasional plasma cells (fig. 14).



Fig. 13.—A small endomysial lesion in a case of myasthenia gravis. The muscle fibers show severe degenerative changes. $\times 250$.

Fig. 14.—A small focal lesion of muscle in a case of fascioscapulohumeral atrophy. $\times 250$.

MISCELLANEOUS DISEASES OF THE CENTRAL NERVOUS SYSTEM AND PERIPHERAL NERVES

This group includes 4 cases of tabes dorsalis, 5 of subacute combined degeneration, 2 of multiple sclerosis, 5 of poliomyelitis varying in duration from one to forty years, 3 in which sympathectomy had been performed, 3 of peripheral neuritis and 1 of injury of the spinal cord. In 2 cases of subacute combined degeneration muscle sections contained diffuse infiltrations of the perimysial tissue, the participating cells being lymphocytes, plasma cells, polymorphonuclear leukocytes and a few, large pale mononuclear cells. Degenerative muscle changes varied from none to severe in this group. In 1 case of peripheral neuritis a section of muscle contained a large focal collection of lymphocytes, with a few large mononuclear and occasional plasma cells. This was located in the perimysial tissue.

MISCELLANEOUS CONDITIONS

Biopsy sections were examined in 43 cases of various conditions. Chronic myositis was present in 1 case of myositis ossificans following trauma, and in 1 case of amputation neuroma. Apart from frank sepsis in 2 cases, no significant lesions were encountered in the remainder.

COMMENT

It is not the purpose in this report to discuss in detail the many lesions which may be encountered in the skeletal muscles in various diseases. An attempt has been made merely to study a variety of sections from routine and selected material with a view to estimating the frequency of occurrence of nonsuppurative chronic inflammatory lesions. Attention was directed in particular to small focal, diffuse or perivascular collections of inflammatory cells and the possibility of distinguishing these from the lesions recently described in rheumatoid arthritis.

It immediately became apparent that such lesions may be encountered in many diseases. They occurred most commonly in dermatomyositis, disseminated lupus erythematosus, periarteritis nodosa and rheumatoid arthritis. No cases of generalized scleroderma were available for study, but similar lesions have been described in that disease.⁶ They have also been reported in cases of rheumatic fever,^{4b} but none was seen in this series.

On the basis of a possible common denominator, this group of diseases has frequently been referred to in the recent literature⁷ as the "collagen diseases." While classic cases in the group are readily differentiated, borderline types may offer considerable difficulty in diagnosis. To resort to biopsy of muscle for purposes of diagnosis in such cases obviously is of doubtful value unless specific and character-

6. (a) Black-Schaffer, B.: *Am. J. Path.* **22**:647, 1946. (b) Weiss, S.; Stead, E. A.; Warren, J. V., and Bailey, O. T.: *Arch. Int. Med.* **71**:749, 1943.

7. Klemperer, P.; Pollack, A. D., and Baehr, G.: *J. A. M. A.* **119**:331, 1942. Banks, B. J.: *New England J. Med.* **225**:433, 1941. *Rheumatoid Arthritis*, *Am. J. Med.* **1**:675, 1946.

istic lesions are present. At best the results of biopsy may be used only as confirmatory evidence to support careful clinical observations.

Lesions which may be indistinguishable on microscopic examination from those observed in the diseases mentioned were found in this series and have also been observed by others, in Addison's disease,⁸ hyperthyroidism,⁹ myasthenia gravis,¹⁰ fascioscapulohumeral atrophy, subacute combined degeneration, peripheral neuritis and subacute bacterial endocarditis. In most of these an inflammatory factor usually is not considered. Diseases in the latter group are etiologically unrelated and clinically more distinct, and offer less difficulty in diagnosis.

An evaluation of the significance of the lesions described is not a simple problem. They do not appear to be specific for any one disease nor even for diseases of common causation. It is unlikely that they represent the presence of a specific infective agent; they probably are related to a profound metabolic disturbance of muscle or collagen tissue. The fact that these lesions are present in muscles which show little evidence of atrophy and absent, in many cases, in muscles showing considerable degeneration, even though the disease is still active and of short duration, indicates the lack of relationship of the two changes.¹⁰

SUMMARY

Inflammatory lesions of skeletal muscle of the type which have recently been described in patients with rheumatoid arthritis may be found in a variety of diseases. A study of tissues obtained in a series of routine and selected cases revealed that such lesions occur in patients with rheumatoid arthritis, dermatomyositis, periarteritis nodosa, disseminated lupus erythematosus and subacute bacterial endocarditis. Indistinguishable lesions were also encountered in cases of Addison's disease, hyperthyroidism, myasthenia gravis, fascioscapulohumeral atrophy, subacute combined degeneration and peripheral neuritis. The lesions were marked by focal, perivascular or, occasionally, more diffuse collections of cells. They were situated in the endomysium with infiltrations extending between individual muscle fibers, or in the perimysium between the muscle bundles. The cells were mainly lymphocytes and a few plasma cells. Polymorphonuclear leukocytes, eosinophils and large, pale mononuclear cells were present in varying proportions. The lesions are regarded as nonspecific in character, and they showed no constant relationship to the degree of degenerative change present in the muscle.

8. Duff, G. L., and Bernstein, C.: *Bull. Johns Hopkins Hosp.* **52**:67, 1933.

9. Thorn, G. W., and Eder, H. A.: *Am. J. Med.* **1**:583, 1946.

10. Mallory, T. B.: *New England J. Med.* **236**:440, 1947.

PLASMA CELL MASTITIS

BÉLA HALPERT, M.D.

JOE M. PARKER, M.D.

AND

JOSEPH M. THURINGER, M.D.

OKLAHOMA CITY

PLASMA cell mastitis was described by Cheattle and Cutler¹ in 1931 and recognized as a clinical and pathologic entity by Adair² and Ewing in 1933. Since then 45 cases of the disease have been formally reported or have been mentioned in discussions.³ In none of these was the mastitis associated with carcinoma of the mammary gland. In 1947 Gaston⁴ reported 3 additional cases of plasma cell mastitis, 2 with unusual complications: One of the patients had a sanguinous discharge of the nipple, and in the other "there was a coexistent comedocarcinoma which had metastasized to the axillary nodes." Within the past two years 3 patients with plasma cell mastitis have come to our attention. In addition, in 3 patients with carcinoma of the mammary gland an inflammatory reaction in which plasma cells predominated was observed, so that the coexistence of the plasma cell mastitis and the carcinoma was seriously considered. The problems of the diagnosis and treatment of plasma cell mastitis are well illustrated in these cases; therefore, our observations are presented in some detail.

REPORT OF CASES

CASE 1.—A 66 year old widow was admitted to the University of Oklahoma Hospitals Sept. 24, 1946. She complained of a mass in the right breast of about three weeks' duration. About three months previously she had fallen through the rungs of a ladder and sustained multiple contusions. She did not, however, recall specifically that her breast was injured. She had three children, all living and well. She passed the menopause at the age of 48.

At the time of admission she was rather obese and appeared younger than her age. The mammary glands were of about equal size and shape. The right nipple was retracted. The surrounding skin had a slight "pigskin" appearance

From the Departments of Pathology, Surgery and Histology and Embryology, University of Oklahoma School of Medicine.

1. Cheattle, G. L., and Cutler, M.: *Tumors of the Breast*, Philadelphia, J. B. Lippincott Company, 1931, p. 298.

2. Adair, F. E.: *Arch. Surg.* **26**:735, 1933.

3. Parsons, W. H.; Henthorne, J. C., and Clark, R. L., Jr.: *Arch. Surg.* **49**:86, 1944.

4. Gaston, E. A.: *Surgery* **21**:208, 1947.

and was reddened and tender over an area 8 cm. in diameter. Beneath the skin and apparently attached to it, a firm, irregular mass about 4 cm. in diameter was felt. In the axilla three slightly enlarged lymph nodes were palpable. Urinalysis gave essentially negative results. The red blood cell count was 4,500,000; the hemoglobin content, 12.5 Gm. The white blood cell count was 10,000, with neutrophils 74 (stabs 4), eosinophils 6, lymphocytes 16 and monocytes 4 per cent. Roentgenologic examination of the chest disclosed no neoplastic involvement. On September 26, the mass was incised and examination made of a frozen section. When a diagnosis of chronic inflammatory reaction and no cancer was returned, simple mastectomy was performed. The operative wound healed, and the patient was discharged October 4. When she was seen about one year later there was no recurrence.

Description of Specimen.—The amputated right mammary gland measured 20 by 16 by 5 cm. and weighed 740 Gm. The skin covering the surface was 19 by 13 cm. The nipple was in the center and level with the skin surface. The areola was inconspicuous. A firm mass, 4 cm. in diameter, was located beneath the areola. From the cut surfaces soft creamy material could be expressed, forming molds 0.2 to 0.4 cm. in diameter.

Microscopic preparations from various parts of the involved area disclosed distended lumens of acini and ducts filled with an amorphous pink material. Other lumens contained many large mononuclear cells with foamy cytoplasm. Still others were almost obliterated. Surrounding the lumens there was dense cellular infiltration, with many plasma cells (fig. 1), some lymphocytes and some large mononuclear cells participating. Elsewhere the loose or more dense connective tissue containing the lumens was densely infiltrated with lymphocytes, many plasma cells, large mononuclear cells with foamy cytoplasm, and occasional giant cells of the foreign body type. In places a necrotic debris contained cellular and nuclear fragments of granulocytes and was surrounded by granulation tissue.

CASE 2.—A 42 year old housewife was admitted to St. Anthony Hospital, Oklahoma City, Sept. 18, 1946. She complained of a lump in the right breast of about six years' duration. The lump was felt particularly during menstruation, and for the past year there had been an intermittent creamy yellow discharge of the nipple during menstruation. She had one child, 7 years old, living and well.

At the time of admission the patient was well nourished and not obese. The right mammary gland was enlarged to almost twice the size of the left. Two masses were palpable: one beneath the nipple, 6 by 5 by 3 cm., and one in the lower half of the gland, 3 by 3 by 2 cm. The masses were firm and easily movable, unattached either to the underlying tissues or to the skin. The skin was not wrinkled but rather appeared somewhat stretched over the masses. The nipple was not distorted. A thick creamy liquid could be expressed. Three nodular areas were felt in the left mammary gland. These were discrete and measured 1 to 2 cm. in diameter. The lymph nodes of both axillas were palpable. Urinalysis gave essentially negative results. The red blood cell count was 4,060,000; the hemoglobin content, 11 Gm. The white blood cell count was 11,300, with neutrophils 70 and lymphocytes 30 per cent. Simple right mastectomy was performed. The patient was discharged on the seventh day following operation with the wound healed. When she was seen about one year later, there was no recurrence of symptoms.

Description of Specimen.—The amputated right mammary gland was rather large and contained beneath the nipple one mass, 6 by 5 by 3 cm., and in its

lower half another, 3 by 3 by 2 cm. Apparently uninvolved mammary gland tissue surrounded the masses. On the cut surfaces these masses had a variegated appearance with creamy and opaque yellow fields. Dilated ducts contained a thick putty-like material which on pressure yielded molds up to 0.4 cm. in diameter.

Microscopic preparations disclosed various-sized, irregularly spaced lumens lined by flat or cuboidal, partly desquamated cells with foamy cytoplasm. Others were filled with a pink-stained amorphous material, streaked and vacuolated. In the surrounding connective tissue there were regions densely infiltrated by lymphocytes, many plasma cells and some large mononuclear cells. In areas composed of adipose tissue there were streaked, amorphous fields resembling fatty acid crystals surrounded by dense aggregations of large mononuclear cells with foamy cytoplasm and occasional giant cells of the foreign body type. Within these areas of fat necrosis there were dense concentrations of lymphocytes and many plasma cells.

CASE 3.—A 51 year old white woman was admitted to the University of Oklahoma Hospitals July 8, 1948. One and one-half years, previously she had noted a clear, watery fluid coming from the nipple of the right breast which lasted for one month, then subsided. She remained asymptomatic until six months prior to admission, when a similar fluid appeared from the left nipple. This discharge also subsided spontaneously after about one month. Three weeks before admission she noted a lump in the left breast and a thick yellow material being discharged from the nipple. She had undergone fifteen pregnancies with three miscarriages and had twelve children.

At the time of admission the patient was well nourished. Both mammary glands were pendulous and of about equal size. Both nipples were retracted. There was no discharge of either. In the upper inner quadrant of the left mammary gland there was a firm, tender, irregularly nodular mass 8 cm. in diameter. The mass seemed not to be fixed to the underlying tissues nor to the overlying skin. The axillary lymph nodes were not palpably enlarged. No masses were present in the right mammary gland. Urinalysis gave essentially negative results. The red blood cell count was 4,820,000; the hemoglobin content, 13.5 Gm. The white blood cell count was 7,350, with neutrophils 76, lymphocytes 23 and eosinophils 1 per cent. The Mazzini test of the blood was negative. Roentgenologic examination of the chest disclosed no neoplastic involvement. On July 12 the mass was incised and examination made of a frozen section. When the diagnosis of chronic inflammatory reaction was returned, simple mastectomy was performed by Dr. Hal A. Burnett. The postoperative course was uneventful, and the patient was discharged July 17.

Description of Specimen.—The amputated left mammary gland measured 19 by 18 by 6 cm. The skin covering the surface was 19 by 13 cm. The nipple was in the center, measured 0.8 cm. in diameter, was depressed 1 cm. below the surface and was surrounded by an areola 2 cm. wide. A firm mass about 8 cm. in diameter was felt in the upper inner quadrant. On the cut surfaces the mass was irregularly circumscribed and contained various-sized spaces up to 1.5 cm. in diameter. These were filled with a debris. There were also mottled gray, yellow and red areas. In the surrounding mammary gland tissue the lumens of ducts were conspicuous and were filled with a putty gray semisolid material which on pressure projected as molds up to 0.6 cm. in diameter.

Microscopic preparations disclosed spacious ducts lined by flat or cuboidal cells. The lumens contained a streaked or vacuolated coagulum. Surrounding the ducts

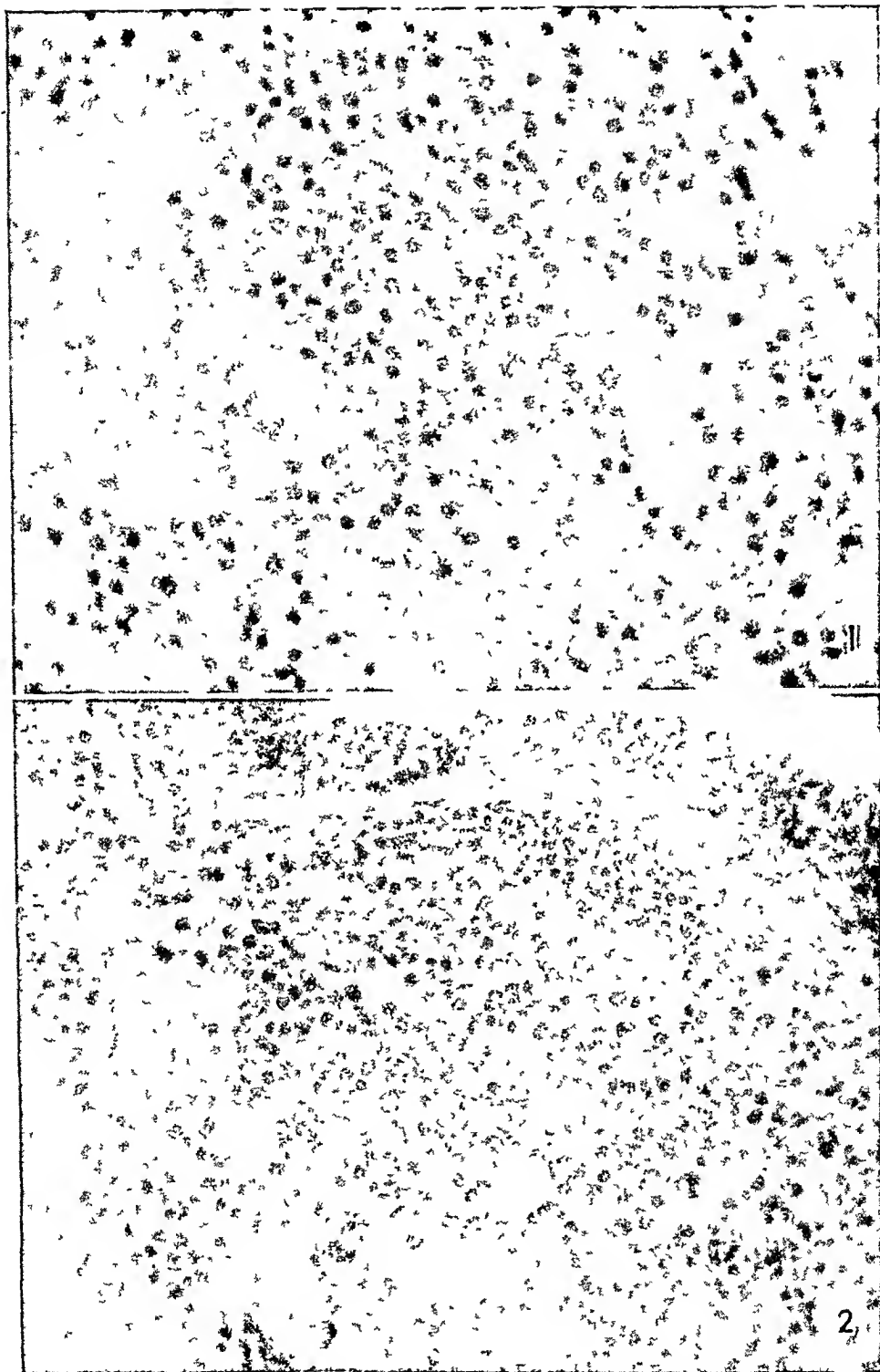


Fig 1—Plasma cell mastitis in a woman aged 66. Surrounding the lumen of a duct there is a dense concentration of plasma cells, some lymphocytes and a few large mononuclear cells $\times 200$

Fig. 2.—Plasma cell reaction in the mammary gland of a Negro woman aged 63 with carcinoma of the mammary gland $\times 200$.

and groups of acini in focal areas there were aggregations of lymphocytes, many plasma cells and occasional large mononuclear cells. Elsewhere an area of necrosis extended into the lumen of a duct which contained many large mononuclear cells, a tissue debris with some neutrophilic granulocytes and their nuclear fragments. A zone of infiltration with many plasma cells and lymphocytes surrounded such an area. Areas of fat necrosis with the usual cellular reaction were more extensive in some preparations than in others.

Case 4 is included with the permission of Dr. Lauren V. Ackerman, pathologist, Ellis Fischel State Cancer Hospital, Columbia, Mo.

CASE 4.—A 63 year old Negro woman was admitted to the Ellis Fischel State Cancer Hospital May 24, 1943. She complained of a lump in the left breast of four months' duration. For the last two weeks prior to admission her breast increased in size and became painful, and she had fever. At the time of admission she was rather obese. The left mammary gland was enlarged, the nipple was slightly retracted and excoriated. There was a *peau d' orange* appearance of the skin around it for a radius of 7 cm. The axillary lymph nodes were not palpably enlarged. A biopsy revealed an inflammatory reaction with collections of plasma cells. A mastectomy with removal of the axillary contents was performed by Dr. H. Everett Sugarbaker. The wound healed without complications. There was no recurrence thirty-eight months later.

Description of Specimen.—The resected left mammary gland contained in its deeper portion a firm mass, 4 by 3 by 3 cm.

Microscopic preparations from various parts of the mass disclosed sheets of neoplastic epithelial cells in a newly formed, loose connective tissue stroma. The cell nuclei were large and vesicular, with a number in varying stages of cell division. About many of the lumens of acini and ducts of the mammary gland there were dense concentrations of lymphocytes, some large mononuclear cells and many plasma cells (fig. 2). The infiltrating plasma cells were usually more conspicuous in the fields not involved in the neoplastic process than in those involved. Two of 29 lymph nodes examined contained tumor metastases.

CASE 5.—A 55 year old woman was admitted to the University of Oklahoma Hospitals Jan. 29, 1947. She complained of an ulcerated lesion of the right breast of three months' duration. Before ulceration a mass had been felt in the right breast for five months. Since then it had increased in size rapidly, and she had progressively lost weight and strength. She was the mother of six children, living and well (ages not available). Menstruation ceased at the age of 44 years.

At the time of admission the patient was thin, cachectic, weighing 92 pounds. In the upper outer quadrant of the right mammary gland there was an area of ulceration, 6 by 5 cm., with elevated and everted hard borders 1 to 2 cm. thick. The right axillary lymph nodes were palpably enlarged. Urinalysis gave essentially negative results. The red blood cell count was 4,450,000; the hemoglobin content, 13 Gm. The white blood cell count was 7,850, with neutrophils 76 (stab forms 17), lymphocytes 18 and mononuclears 6 per cent. The Mazzini test of the blood was negative. Roentgenologic examination of the chest disclosed no pulmonary involvement. Biopsy of the border of the ulcer revealed carcinoma. On February 5, amputation of the right mammary gland was performed by Dr. Harrell C. Dodson. The postoperative course was uneventful, and the patient was discharged on February 17, with the operative wound healed.

Description of Specimen.—The amputated mammary gland measured 17 by 11 by 7 cm. and weighed 420 Gm. The nipple was in the center of the elliptic portion of skin, 17 by 10 cm. It measured 1 cm. in diameter and appeared raw.

Upward and laterally to the nipple there was an ulcerated area, 6 by 4 cm. The margins of the defect in the skin were elevated and firm. Underlying this region and the nipple, there was a firm mass, 5.5 by 5 by 3 cm. On the cut surfaces the mass appeared sunken below the level of the surrounding tissue and was gray-white, dotted with opaque and fibrillar areas. Strands of gray-white tissue radiated from the mass into the surrounding tissue. In the nearby adipose tissue several globular masses, 0.7 to 2 cm. in diameter, were located. These were identified as lymph nodes with neoplastic involvement.

Microscopic preparations from the mass disclosed sheets of neoplastic epithelial cells having large, vesicular, deeply stained nuclei with some in varying stages of cell division. The cell nests were within a loose or more dense, hyalinizing fibrous connective tissue stroma. In the central portions of the large cell nests there were extensive areas of necrosis. The neoplastic cells were seen invading and replacing adipose tissue. Within the connective tissue stroma there were areas infiltrated by lymphocytes, some large mononuclear cells and many plasma cells. About the mammary gland acini and ducts and in streaks within the stroma elsewhere there were dense plasma cell infiltrations. In preparations from the lymph nodes only streaks of lymphatic tissue remained; the rest was replaced by neoplastic cells and their stroma.

CASE 6.—A 60 year old white woman was admitted to the University of Oklahoma Hospitals Nov. 3, 1947, complaining of a mass in the left mammary gland. In 1937 there had been some discharge of the nipple. At that time she noticed a mass about 2 cm. in diameter in the same mammary gland. After about six years the mass began to increase in size. In July 1947, the growth was almost 10 cm. in diameter, and erosion of the skin began and progressed toward the nipple. The patient had undergone ten pregnancies with six miscarriages and had four children living and well.

At the time of admission she was slightly obese, with no obvious loss of weight. The mammary glands were large and pendulous, with the left somewhat larger than the right. Underlying a granular excoriated area of the skin 2 cm. in diameter involving the nipple and extending upward and laterally, there was a hard, irregular mass, 8 by 8 cm. The mass was in the outer portion of the mammary gland and not attached to the chest wall. The axillary lymph nodes were not palpable. There were no masses in the right mammary gland. Examination of the urine gave essentially negative results. The red blood cell count was 3,650,000; the hemoglobin content, 12 Gm. The white blood cell count was 11,800, with neutrophils 65, lymphocytes 23, monocytes 10 and eosinophils 2 per cent. The Mazzini test of the blood was negative. Roentgenograms of the chest revealed no pulmonary metastasis. On November 5, after a diagnosis of carcinoma was returned on examination of a frozen section, radical mastectomy was performed by Dr. Harrell C. Dodson. When the patient was seen March 3, 1948, there was no recurrence.

Description of Specimen.—The amputated left mammary gland weighed 2,160 Gm. An elliptic portion of skin, 24 by 14 cm., covered the anterior surface. The nipple was located near the center. It was retracted to 0.2 cm. above the surface and was excoriated, with the areola inconspicuous. Beneath the nipple there was a firm mass, 6 cm. in diameter, which on the cut surfaces appeared sunken below the level of the surrounding tissues with streaks of gray-white tissue radiating from it. In the remaining portions of the mammary gland there were lumens up to 0.4 cm., yielding putty-like molds on pressure.

Microscopic preparations from the mass disclosed sheets and nests of neoplastic epithelial cells with vesicular or compact, deeply stained nuclei in a pink-stained

or halo-like cytoplasm, within a connective tissue stroma. Elsewhere the cells were within tissue or lymph spaces. Surrounding the remains of mammary gland acini and ducts and also about the neoplastic cell nests there were dense concentrations of lymphocytes, many plasma cells and some large mononuclear cells. Necrosis was seen within the neoplastic areas. Areas of fat necrosis were absent. Ten lymph nodes examined were free of neoplastic involvement.

COMMENT

Plasma cell mastitis may be redefined as a focal chronic inflammatory process in the mammary gland. Plasma cells are dominant in the inflammatory process and far outnumber all the other cells participating in the reaction. The cause of the lesion is as yet unknown.

Multiple areas of fat necrosis often accompany plasma cell mastitis. In our 3 cases of plasma cell mastitis there were areas of fat necrosis. Either fat necrosis as such or plasma cell mastitis alone or both in combination form a solid mass, indefinitely delineated, not unlike carcinoma.

In our 3 cases of carcinoma of the mammary gland there was a cellular reaction in which plasma cells predominated. The fact, however, that most of the cellular reaction was about the acini and ducts of the mammary gland and in areas distant from the neoplastic involvement favors the view that the plasma cell reaction was independent of the carcinoma.

An accurate history may aid in the differential diagnosis. A firm mass appearing in the mammary gland following trauma and persisting for years with no appreciable change in size suggests fat necrosis. A firm mass appearing in the mammary gland without a history of trauma and persisting for years with no appreciable change in size suggests plasma cell mastitis. A sudden increase in the size of either type of mass points toward a combination with carcinoma. In any event the nature of the mass can be determined only by microscopic examination. Diagnosis by frozen section, however, can be accurate only if a representative portion of tissue is examined. Radical operation is indicated only in case the mass proves to be cancerous.

SUMMARY

Plasma cell mastitis is defined as a focal chronic inflammatory process in the mammary gland, frequently associated with areas of fat necrosis. Plasma cells are dominant in the inflammatory process and far outnumber all the other cells participating in the reaction. The cause of the lesion is as yet unknown.

Clinical data are presented, concerning 3 patients with plasma cell mastitis and 3 patients with carcinoma of the mammary gland in whom there was a cellular reaction with plasma cells predominating about acini and ducts believed to be independent of the carcinoma.

A QUANTITATIVE APPROACH TO THE STUDY OF SPLENOMEGALY

ALVIN J. GORDON, M.D.

ERNEST C. HOLDER, M.D.†
AND

SERGEI FEITELBERG, M.D.
NEW YORK

STUDIES of the morbid anatomy of splenomegaly have hitherto been confined to an analysis of the general structure of the spleen and the finer cytologic details. Approximate estimates of the percentages of white pulp, red pulp and trabecular system are a corollary to an appraisal of the lesion. Because of the obvious inaccuracy of such haphazard estimates, it was thought that an exact quantitative determination of the constituents of the spleen might yield information regarding the nature and the evolution of the morbid process.

In general, enlargement of any organ can be conceived as the response to a stimulus. Enlargements occurring under morbid conditions can be classified into different groups according to the textural alterations which can be recognized by anatomic-histologic investigation. Broadly speaking, an organ may increase in size if it is the seat of inflammation or of neoplastic growth, or if its constituent elements increase in number (hyperplasia) or in size; the latter type of enlargement may be due to hypertrophy of the organ or to its being infiltrated with products of metabolism.

The same principle of morphogenetic classification can be applied to enlargements of the spleen. However, the identification and the separation of the individual groups are far more complicated in the case of the spleen than in that of any other organ of the body. This difficulty is partly explained by the intricacy of the splenic structure and the complexity of its cellular constituents under normal and particularly under morbid conditions. It is accounted for, furthermore, by the observation that the basic cellular constituents of the spleen, which can adequately be designated as the parenchyma of the organ, react with similar proliferation to dissimilar stimuli. A pathogenetic appraisal of splenic enlargement can therefore not be founded on the simple

† Deceased.

From the Laboratories (Departments of Pathology and Physics), Mount Sinai Hospital.

morphogenetic analysis which applies to other organs. It must correlate the relative augmentation of the various cellular elements with the respective alteration of the splenic structure observed in splenomegalies of obviously different, but defined, etiology. In this manner criteria have been established which permit one to make a tentative morphologic classification of splenomegalies on a dynamic basis.

As in any other organ, so in the spleen structure and function are mutually dependent. The main splenic functions can be divided into those of metabolism (including defense against infection), blood storage and blood cell formation. The reservoir function of the spleen is obviously associated with the structural pattern of the red pulp, while the formation of blood cells seems to be divided between the red and the white pulp. Which of these portions is more prominently engaged in defense reactions is not so obvious from a consideration of the normal spleen. Alterations of the splenic structure must reflect alterations of function, so that it seems justified to assume that a prominent enlargement of one of the main constituents of the spleen (i. e., red and white pulp) can be regarded as the result of an increased functional stimulation. For this reason the quantitative analysis of various forms of splenomegaly appeared to be indicated.

It was obvious that first such forms had to be selected in which the pathogenic factor responsible for enlargement was clear. It was to be expected that in some of these groups the results of the laborious measurements could have been anticipated from the gross appearance of the spleen. But by proceeding in these investigations from the known to the unknown, it was hoped that observations could be made which might indicate which of the splenic partitions was prominently involved. Such results could then point the way to inclusion or exclusion of certain factors hitherto prominently considered in the explanation of the pathogenesis of the particular form.

This report is concerned with the modification of a method for the quantitative estimation of the percentages of white pulp, red pulp and trabeculae, together with a consideration of the results obtained in a group of representative splenomegalies. In the mathematical analysis of the material, measurements had to be made of the white pulp and of the trabeculae, and the amount of red pulp was then arrived at by subtraction.

It was apparent at the outset that many difficulties would arise and that the results might not be statistically significant.

The pioneer work in this field was done by Hellman,¹ who obtained the percentage composition of normal spleens at various ages. He measured white pulp, red pulp and stroma in 100 cases of sudden death.

1. Hellman, T.: *Ztschr. f. Konstitutionslehre* 12:270, 1926.

Under the stroma he included trabeculae, capsule and blood vessels. He also measured the area of the germinal centers (secondary follicles).

His method was to project microscopic fields onto paper of uniform composition and thickness, trace and cut out the various components, and determine their percentages by weighing the paper.

Under the white pulp he included the malpighian corpuscles and perivascular lymph sheaths. His percentage weights for white pulp and connective tissue are shown in table 1.

Using a technic similar to Hellman's, von Herrath² obtained the percentages of the various constituents of spleens of various mammals, including man. Only 22 spleens were thus examined, 2 of which were human. The figures are therefore statistically not significant. For

TABLE 1.—*Percentage Weights of the White Pulp and the Connective Tissue as Determined in Normal Spleen by Hellman*

Age Group	White Pulp	Connective Tissue
Fetuses.....	9.55	1.97
Newborn infants.....	10.69	3.37
0-1 yr.....	20.95	4.03
2-5 yr.....	21.49	5.74
6-10 yr.....	19.63	6.20
11-15 yr.....	15.66	6.46
16-20 yr.....	13.67	5.97
21-30 yr.....	9.62	6.91
31-40 yr.....	9.83	7.82
41-50 yr.....	8.27	9.58
Over 50 yr.....	6.43	11.07

TABLE 2.—*Percentages of the White and the Red Pulp and the Trabeculae as Observed in Two Human Spleens by von Herrath²*

Case	White Pulp	Red Pulp	Trabeculae
1.....	18.93	74.01	7.06
2.....	17.45	78.03	4.47

purposes of comparison, however, the results which he obtained in his 2 human cases are quoted. (The ages of the patients were not stated.)

Hwang, Lippincott and Krumbhaar³ studied the percentage areas of white pulp in 300 normal human spleens of various ages. Their particular purpose was to check the correlation between white pulp and age. They also counted the number of follicles per unit area and measured the areas taken up by the pale-staining centers of follicles plus the central arteries. Their method was apparently less cumbersome than that of the previous authors. They projected the microscopic field onto a piece of paper, traced the limits of the tissues to be estimated, and measured the areas with a planimeter.

2. von Herrath, E.: *Ztschr. f. mikr.-anat. Forsch.* **37**:389, 1935.

3. Hwang, J. M. S.; Lippincott, S. W., and Krumbhaar, E. B.: *Am. J. Path.* **14**:809, 1938.

They found that the amount of lymphatic tissue (including germinal centers and central arteries) was small in infants (4.8 per cent up to 1 year of age), rose to a maximum in the first decade (12.1 per cent), then dropped sharply (8.6 per cent, the mean for the decade 11 to 20) and continued at about that level (chart 1). After subjecting their results to statistical analysis, they stated that if the groups from 11 years on were considered, the line was not significantly different from horizontal, although it did have a slightly downward trend. They detected the suggestion of an increase in the percentage of lymphatic tissue in the sixth and seventh decades, with a drop thereafter.

It is evident that certain correlations exist between the age and the percentage composition of the spleen. If in our studies the abnormal spleens were to be compared with controls of the same age group, the

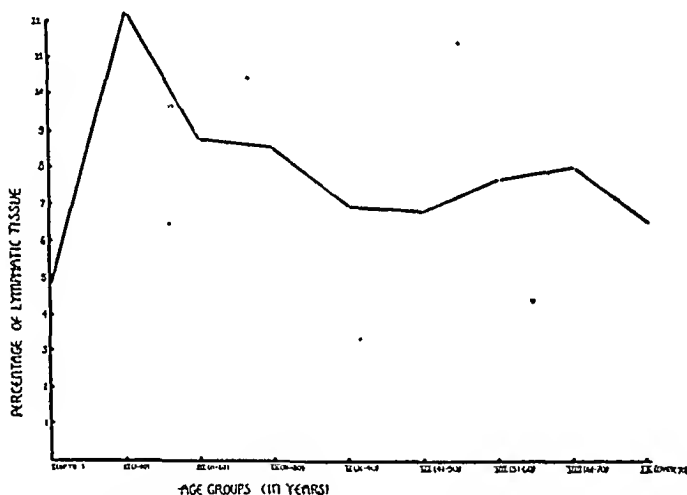


Chart 1.—Percentage of follicular lymphatic tissue at various ages (after Hwang, Lippincott and Krumbhaar³).

amount of work involved would be prohibitive, and the material available insufficient. Therefore, since the greatest age variations exist in childhood, we sought to determine whether, by using only subjects over 21, it would be valid to pool all the ages in the final computations.

The answer was in the affirmative. It has already been mentioned that Hwang and associates³ found that the percentage of lymphatic tissue was relatively constant for the years over 11. As their paper did not include all the figures, this conclusion could not be checked. However, using Hellman's¹ statistics, we determined that for the ages over 21 there was no correlation between age and the percentage of white pulp. (The correlation coefficient was 0.28, with a standard error of 0.12.)

A different situation existed in regard to the connective tissue, for there Hellman's figures showed an increase with age (table 3, adapted

from his table 24). This point must be borne in mind in interpreting the final results of our calculations. However, as will be seen subsequently, the average ages of our controls were not markedly different from those of the patients with splenomegaly. The error from this source is therefore minimized.

MATERIAL

Normal Spleens.—Thirty “normal” spleens were obtained through the courtesy of Dr. Milton Helpern and Dr. Jacob Werne, Medical Examiners, New York. They were obtained in cases of sudden (violent or accidental) death in which no

TABLE 3.—Percentage Areas of Connective Tissue Determined for Age Groups by Hellman¹

Age Group	Cases	Connective Tissue, per Cent
21-30.....	23	6.91
31-40.....	17	7.82
41-50.....	12	9.58
Over 50.....	4	11.07

TABLE 4.—Cases of Splenomegaly Included in This Study, Divided into Classes According to Diagnoses

Diagnosis	Cases	Average Age, Yr.	Average Wt. of Spleen, Gm.
Subacute bacterial endocarditis.....	15	43	500
Laennec's cirrhosis (uncomplicated by disease of splenic or portal vein)	17	47	513
Laennec's cirrhosis complicated by thrombosis, thrombophlebitis, phlebosclerosis or carcinomatous invasion of portal or splenic vein.....	9	54	619
Obstruction of splenic or portal vein without cirrhosis of the liver (thrombosis, phlebosclerosis, cavernomatous transformation or extrinsic pressure on the portal or splenic veins)	8	53	731
Leukemia (acute and chronic myeloid types).....	8	48	1,691
Chronic cardiac failure.....	6	48	410
Polycythemia vera (1 case with thrombosis of portal, splenic and hepatic veins).....	5	59	1,239
Gaucher's disease	3	39	1,333

history or autopsy findings were present to suggest any antecedent disease. The subjects were all over 21 years of age, were of both sexes and belonged to the white and the Negro race. No attempt was made to segregate them according to sex or race. Body weight was not considered. The average age of these controls was 43; the mean weight of the spleen was 128 Gm.

Diseased Spleens.—Both postmortem and surgical material was used. No cases were included in which the diagnosis was in doubt. A specimen removed at operation was utilized only if the diagnosis was either clear at operation or subsequently confirmed by postmortem examination. All the patients were over 21 years of age. No distinction of sex or race was made. Table 4 summarizes these details of the included cases.

It is apparent that, whereas some of the groups are fairly homogeneous, others vary considerably in their pathogenesis. In general, an attempt was made to

include in each group diseases which would be expected or are known to exert similar effects on the spleen.

Subacute bacterial endocarditis was chosen as the prototype of a chronic infectious disease, while obstruction of the portal or the splenic vein was considered representative of the group ascribed to hemodynamic influence.

As far as possible, an attempt was made to exclude cases in which the primary disease was complicated by a pathologic process which might have an effect on the spleen. As an example, the presence of long-standing cardiac failure would exclude from consideration a case of subacute bacterial endocarditis.

Because the choice of spleens was limited, it was impossible to take into consideration the duration and the extent of the disease process.

The relatively small number of cases included in each group was dictated both by the paucity of available material and by the time-consuming nature of the work. To this one factor alone (i. e., the paucity of samples) are probably attributable the failure to obtain results of statistical significance in many instances. However, in regard to certain diseases, such as polycythemia vera and Gaucher's disease, it was only necessary to examine a few spleens to obtain significant figures. This could be predicted in advance by a consideration of the extensive changes in the spleen.

METHODS

Usually a large section of each normal spleen was taken at postmortem examination, through the length of the organ and perpendicular to its convexity, and fixed in formaldehyde solution. The specimens of pathologic spleens were taken from material fixed in Kaiserling's solution and usually cut originally as just stated.

From each specimen, three flat slices were cut, measuring about 2 to 3 mm. in thickness. The dimensions of each such section were about 1.5 by 3.5 cm. An attempt was made to take one slice from each pole and one from the center of the spleen. This was not always possible. The sections usually included a portion of splenic capsule at one edge.

Paraffin sections were stained by the hematoxylin-eosin method. Each section was placed on a separate slide, so that there were three slides for each spleen.

The percentage areas of trabeculae and white pulp were measured, and the red pulp made up the difference.

The method was a modification of that used by Hwang and associates.³

These authors projected the microscopic field onto a piece of paper, outlined the white pulp and measured the tracings with a planimeter.

In our method one step was eliminated by projecting the field from below onto a piece of translucent paper fixed to a large plate of glass acting as a table. The image could thus be measured directly on the paper without first tracing the outlines.

A horizontally placed carbon arc microscope projector was used, placed on a platform 10 inches (25.5 cm.) high, which rested on the floor. By means of a prism, the image was reflected upward, coming to a focus on a sheet of "onion skin" paper fixed by Scotch tape on the top of the plate of glass. The latter was 31 inches (76 cm.) from the floor. The work was done in a darkened room, and the image thus obtained was clear enough for the purpose. The light traversed the glass plate from below, and the field could be measured directly on the paper from above.

For each area measured it was necessary only to make one dot with a pencil on the periphery as a point of reference for the planimeter. The translucent paper was changed frequently.

The "geographic" objective was used (22.4 mm., 6 ×) and the 10 × ocular. The final magnification was about 90 diameters. The actual diameter of the final image was 8 5/16 inches (21 cm.). All distances were fixed, and thus the magnifications were constant.

To retard overheating and fading of the slide, the source of light for the microscope projector was passed through a glass container of chopped ice and water, which was changed frequently. Despite this, considerable fading and distortion occurred.

In each case, 16 fields were counted, each field representing approximately 0.0066 square inch (0.04 sq. cm.) of spleen; the total was therefore 0.10 square inch (0.65 sq. cm.).

The reasoning by which it was determined to calculate 16 fields was as follows: Using Hellman's figures for white pulp and trabeculae, divided into age groups by decades, we found that the standard deviation for each case reached as high as 40 per cent. With this expected variation, therefore, it was considered that our own accuracy need not be better than 30 per cent. One of our cases was then taken as a sample, and the percentages of trabeculae and white pulp determined by measuring 20 fields. From these figures it was then possible to determine the standard deviation, and it was calculated that a standard error of 30 per cent for the trabeculae and 15 per cent for the white pulp would obtain when 16 fields were measured. As the later computations showed, an accuracy of better than 30 per cent was actually achieved in each case.

As it had been shown previously that the malpighian corpuscles are uniformly distributed throughout the spleen,⁴ and that the same probably holds true for the trabeculae,² the 16 fields were taken at random, except that the capsule was avoided. Usually 5 or 6 fields were measured in each of the three sections.

In measuring lymphatic tissue, considerable difficulty was encountered, as was expected, and the error therefore was considerable. All aggregates of lymphocytes were measured which were sufficiently large and concentrated to give a distinct blue area on the screen. Most of these areas were malpighian corpuscles and collections about blood vessels, but occasionally isolated collections of cells also occurred. Many of the latter probably represented follicles cut through their peripheries.

More often than not the limits of the lymphatic tissue were not sharp and the borders taken were arbitrary ones. Such borders were usually taken around the periphery of the dense zone. The measurements were all done by the same person and in the same fashion, so that the error from this source was minimized.

No attempt was made to separate the germinal centers from the remaining lymphatic tissue. When blood vessels and their adventitial sheaths within lymphatic collections amounted to more than half of the area in question, they were included with the trabeculae rather than the lymphatic tissue. This, of course, was also an arbitrary rule.

Unavoidable errors were introduced by artefacts, such as shrinkage, slight cracking and the separation of the tissue sections.

The measuring of the trabeculae was easier, because the borders were usually well defined. Blood vessels larger than capillaries were included with the trabeculae.

4. Hellman.¹ Hwang and others.³

STATISTICAL TREATMENT

Individual Spleens.—For each of the sixteen fields the area of white pulp and that of trabeculae were obtained separately. The mean area per cent was then calculated. The standard deviation of each measurement and the standard error were then obtained by means of the formulas:

$$\sigma = \sqrt{\frac{\sum (s^2)}{n-1}} \quad (1)$$

$$\sigma_e = \frac{\sigma}{\sqrt{n}} \quad (2)$$

where

σ = the standard deviation of a single measurement

s = the difference of each measurement from the arithmetic mean

n = the number of cases

σ_e = the standard error (which is also referred to as standard deviation of the mean)

The final figures for an individual spleen would then appear, for example:

Trabeculae	3.99% \pm 0.6% (standard error)
White pulp	9.25% \pm 1.16
Red pulp	100-3.99-9.25 = 86.76%

Groups of Spleens.—For each group (i. e., normal spleens, spleens obtained in cases of Laennec's cirrhosis, etc.) the percentages were totaled, the mean percentage obtained for the entire group, and the standard deviation obtained by the formula⁵:

$$\sigma = \sqrt{\frac{\sum (X)^2}{n} - \bar{X}^2} \quad (3)$$

where

σ = the standard deviation

n = the number of cases in the group

$\sum (X)^2$ = the sum of the squares of each unit

\bar{X} = the mean for the group.

The standard error was then obtained from the standard deviation by equation 2.

5. This formula is more convenient for calculation of the standard deviation in a larger number of cases (see Arkin, H., and Colton, R.: *An Outline of Statistical Methods*, New York, Barnes & Noble, 1947, p. 33).

Each group of abnormal spleens was then compared with the control group to determine whether the differences were statistically significant. These calculations involved the white pulp and trabeculae. First, the percentage areas were compared. The difference between the abnormal and the control was taken, and the ratio of this difference to the standard

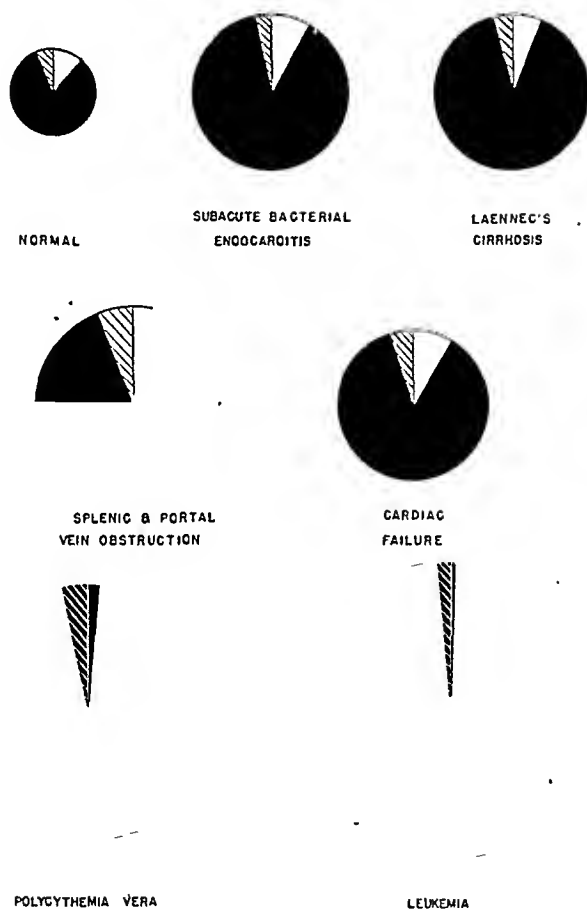


Chart 2.—Diagrammatic representation of the results given in table 5. The area of each circle is proportional to the weight of the spleen. The solid black represents red pulp; the cross-hatched area, trabeculae, and the clear area, white pulp.

error of the difference was calculated (ratio of significance). The standard error of the difference, σ_d , is calculated from the formula:

$$\sigma_d = \sqrt{\sigma_1^2 + \sigma_2^2} \quad (4)$$

where

σ_1 = the standard error of the value for the controls

σ_2 = the standard error of the value for the pathologic specimens

TABLE 5.—*Percentage Composition of Spleens Obtained in Cases of Splenomegaly Compared with That of Normal Spleens*

Diagnosis	Percentage			Absolute Weight												
	Tra-been-lae		White Pulp	S.R.*	Red Pulp	Mean Wt.	Index†	Tra-been-lae	S.R.	Index	White Pulp	S.R.	Index	Red Pulp	Index	
	Cases	6.21														
Normal.....	30	6.21	11.99	81.80	123	...	7.95	15.32	105.0	...
Subacute bacterial endocarditis.....	15	2.79	2.59	8.50	1.11	88.71	500	3.9	13.05	1.47	1.75	42.50	2.3	2.7	443.5	4.2
Laennec's cirrhosis (simple)...	17	3.89	1.53	6.22	2.11	89.89	513	4.0	20.00	2.0	2.50	31.95	1.76	2.1	462.0	4.4
Laennec cirrhosis with disease of splenic or portal vein.....	9	7.29	0.34	5.10	2.30	87.61	619	4.8	45.10	2.16	5.6	31.50	1.34	2.1	542.5	5.2
Obstruction of splenic or portal vein.....	8	5.91	0.04	2.48	3.96	91.61	731	5.7	43.25	2.05	5.4	18.13	0.38	1.2	670.0	6.4
Leukemia.....	8	1.44	3.82	0.13	5.31	98.43	1,691	13.2	24.40	1.56	3.0	2.20	6.26	0.1	1,694.0	15.9
Cardiac failure.....	6	5.09	0.19	9.25	0.67	85.66	410	3.2	20.90	1.36	2.6	37.95	1.30	2.5	351.8	3.4
Polycthemia vera.....	5	3.27	1.51	1.50	4.52	95.23	1,239	9.67	40.50	1.59	5.1	18.60	0.35	1.2	1,180.0	11.2
Gaucher's disease.....	3	3.02	1.44	2.04	3.77	94.94	1,333	10.4	40.30	1.31	5.0	27.20	0.69	1.8	1,263.0	12.0

* S. R. = significance ratio.

† Index = ratio of weight of spleen to that of a normal spleen.

For example, if the white pulp of the controls is compared with the white pulp of the specimens obtained in cases of subacute bacterial endocarditis (table 5):

$$\text{Ratio of Significance} = \sqrt{\frac{11.99 - 8.50}{(2.23)^2 + (2.21)^2}} = 1.11$$

It is an elementary theorem of statistics that the probability that the difference between any two sets of results is significant increases as the foregoing ratio rises. If the ratio is 3, the odds are 369.4 to 1 that the difference is significant. When the ratio is less than 1, the difference is not significant. Between 2 and 3 the odds rise rapidly (Pearl⁶).

A further comparison was obtained by approximating the absolute weights of trabeculae, white pulp and red pulp by multiplying the total spleen weights by the percentages of each constituent. The "absolute weights" of the abnormal spleens were then compared statistically with the weights of the normal controls, and the ratio of significance was obtained.

This, of course, is only a rough method of estimating the relative weights of the various constituents of the spleen, because the volumes of the different parts are not directly proportional to the area measured on a cross section. Furthermore, the specific gravities of the morphologic

TABLE 6.—*Comparison of Means Obtained for Components of Normal Spleens by Different Investigators*

	Connective Tissue	White Pulp	Red Pulp
Our figures (30 cases).....	6.21%	11.99%	81.80%
Hellman ¹ (59 cases).....	8.20	9.36	82.44
Hwang, Lippincott and Krumbhaar ² (253 cases)....	7.32	

units are not taken into account. The aforementioned figures are therefore only an estimation for purposes of comparison. The results appear in table 5 and chart 2.

SOURCES OF ERROR

Because of several unavoidable sources of error, it was early postulated that in many cases no results of statistical significance would be obtained. These sources of error were (1) the great individual variation of normal spleens,⁴ (2) the difficulties of preparing, sampling and measuring the specimens and (3) the small number of cases examined.

PERCENTAGES FOR NORMAL SPLEENS

The only authors who had comparable statistics were Hellman¹ and Hwang and associates.³ In table 6 the means of all spleens taken from

6. Pearl, R.: *Introduction to Medical Biometry and Statistics*, ed. 3, Philadelphia, W. B. Saunders Company, 1940.

persons 21 years of age and over recorded in their tables are compared with ours.

In regard to the white pulp, our computation is higher than either of the others. The explanation of this discrepancy probably lies in the fact that we included small aggregations of lymphatic tissue which the others did not consider significant enough to measure. The other source of difference lies in the points chosen for the borders of the follicles. The difference between our percentage of connective tissue and Hellman's probably occurred because he included a portion of capsule.

PERCENTAGES FOR PATHOLOGIC SPLEENS

As is evident from table 5, significant differences from the normal spleens were observed in some cases, and in certain others the results showed no significant differences. In the great majority, however, the results were of doubtful significance, and hence in the following broad interpretation some reservations must be made.

Subacute Bacterial Endocarditis.—The percentage of trabeculae decreases to 2.79 from a normal of 6.21. The ratio of significance is 2.6, indicating that the odds are 106.3 to 1 against this difference being due to chance. As the average age of these spleens is the same as that of the controls, the age factor need not be considered.

The percentage of white pulp is 8.50, compared with a normal of 11.99, which gives a ratio of significance of 1.1. This means that the odds are only 2.69 to 1 against this difference being due to errors of sampling and determination. In other words, the percentage of white pulp does not differ significantly from the normal.

The red pulp comprises 88.71 per cent of the area, compared with the normal 81.80 per cent.

Summary: In this disease the increase of the weight of the spleen is caused by a growth of both the red and the white pulp, but the trabeculae do not keep pace with this growth.

Laennec's Cirrhosis (Uncomplicated).—The percentage of trabeculae is 3.89, compared with a normal of 6.21. The ratio of significance is 1.5, which allows no definite conclusions to be drawn.

The percentage of white pulp is 6.22, with a ratio of significance of 2.11. This comes under the heading "probably significantly decreased."

The red pulp is increased to 89.89 per cent.

Summary: In this disease there is growth of all elements; the red pulp most, the white pulp least. The final ratio indicates some decrease of the percentages of trabeculae and white pulp as compared with normal spleens, with an increase of the percentage of red pulp.

Laennec's Cirrhosis with Pathologic Involvement of Veins.—The trabeculae amount to 7.29 per cent; the ratio of significance is 0.34, which means that the percentage does not differ significantly from the normal. The white pulp is 5.10 per cent, with a ratio of significance of 2.36; hence it is probably significantly decreased.

The red pulp is increased to 87.61 per cent.

Summary: There is growth of all elements, least of the white pulp. The final ratio indicates no change in the percentage of trabeculae, and a definite decrease in the percentage of white pulp. •

Laennec's Cirrhosis With and Without Pathologic Involvement of Veins.—The results are essentially the same as in the previous group. (The figures are not shown in table 5.)

Pathologic Involvement of Splenic and Portal Veins.—The trabeculae total 5.91 per cent, with a ratio of significance of 0.04—indicating no significant difference from the normal of 6.21.

The white pulp measures 2.48 per cent, significantly decreased from the normal. The significance ratio is 3.96—a high figure.

The red pulp is increased to 91.61 per cent.

Summary: The increase in weight is due to a growth of trabeculae and red pulp. The white pulp does not grow at all. This is clearly brought out by comparison of the absolute weight of the white pulp (18.13 Gm., compared with the normal of 15.32).

Leukemia.—The figure of 1.44 per cent for trabeculae (significance ratio, 3.82) shows a marked decrease. The white pulp amounts to only 0.13 per cent (significance ratio, 5.31), which means that it practically disappears. This, of course, could have been predicted in advance.

The red pulp rises to 98.43 per cent. (As the average age of the leukemic persons was higher than that of the normal ones, and the trabeculae therefore would be expected to increase, the age factor can again be disregarded.)

Summary: The growth is limited to the red pulp; the white pulp disappears; the trabeculae do not take part in the growth. The absolute weight of the trabeculae remains essentially unchanged.

Cardiac Failure.—The ratios show that the percentages do not differ significantly from normal. There appears to be a proportional growth of all elements.

Polycythemia Vera.—The trabeculae total 3.27 per cent, with a ratio of significance of 1.54. The difference is therefore not significant statistically.

The white pulp drops sharply to 1.50 per cent, indicating a significant decrease from the normal of 11.99 per cent.

The red pulp is high—95.23 per cent.

Summary: The growth is chiefly of the red pulp; there is some growth of trabeculae; the white pulp does not participate in the growth. This is reflected in a significant decrease of the ratio of white pulp, some decrease of that of the trabeculae and an increase of the percentage of red pulp.

Gaucher's Disease.—The result of 3.02 per cent for trabeculae is not definitely significant statistically (significance ratio, 1.44). The white pulp drops significantly to 2.04 per cent (significance ratio, 3.77). The red pulp therefore increases—to 94.94 per cent.

Summary: The results are the same as in polycythemia vera.

COMMENT

It is now possible to examine and perhaps answer some of the questions raised by this investigation. Has anything been discovered about the behavior of the spleen, by this quantitative approach, which was not known before?

The answer to this fundamental question is in the affirmative. When more detailed results are sought, however, it is difficult to know where fact ends and speculation begins.

As indicated previously, the problem is one of a statistical analysis in which the sample is perforce relatively small. This discussion is therefore of a tentative nature and calculated more to provoke further investigation along the same lines than to give unequivocal conclusions.

It can certainly be inferred that the spleen does not increase in size as a whole; that is, its elements do not grow proportionately. This is not a startling conclusion of itself and could have been predicted from a knowledge of the histologic aspects of splenomegaly. However, it has now been demonstrated in quantitative terms.

Since the trabecular system serves a purely supportive function (an assumption borne out by our results), it can profitably be overlooked in the present discussion. From this point of view, splenomegaly is caused in the great majority of cases by growth of the red pulp. In only one disease under study, subacute bacterial endocarditis, do the white pulp, the red pulp and the trabeculae show proportional growth. Indeed, study of the estimated absolute weights of the various constituents (table 5) indicates that in obstruction of the splenic or the portal vein and in polycythemia vera, for example, the white pulp remains completely untouched, as if it were an entirely different organ.

The conclusion is inescapable that the structural units respond independently. There are two corollaries to this: 1. If one interpreted a given disease in terms of these units and what is known of their function one might shed light on the nature of the morbid process. 2. Reasoning

in the reverse direction, one might expect the known nature of certain disease processes to increase knowledge of the functions of the individual constituents of the spleen.

Leukemia and Gaucher's Disease.—As expected, the splenic enlargement in myeloid leukemia is due to an overwhelming growth of the red pulp. There is an actual decrease of the total amount of white pulp, indicating that either it has been destroyed or it has failed to regenerate as it would under normal conditions. The overgrowth of red pulp is easily understood in this disease, in which the reticulo-endothelial system becomes involved by virtue of its potentialities as embryonal mesenchyme.

A comparative quantitative study of chronic lymphatic leukemia was not undertaken, because the results could be predicted, and, furthermore, because there was no probability that the malpighian follicles could be differentiated from the red pulp diffusely infiltrated by lymphocytes.

The results in Gaucher's disease are similar to those in leukemia with the one exception that the white pulp fails to disappear. The higher percentage of trabeculae in Gaucher's disease may be a function of the more chronic course of the illness. The results leave no doubt that the red pulp is the site of prominent enlargement by virtue of its metabolic activity.

Polycythemia Vera.—The spleen in polycythemia vera has been described by Delannoy⁷ as showing a striking engorgement of the sinuses and a conspicuous cellularity of the red pulp, with but few malpighian corpuscles. Klemperer⁸ noted hyperplasia of the cytoplasmic reticulum of the red pulp. The quantitative measurements reveal a significant decrease in the percentage of the white pulp, although the total amount is not as markedly diminished as in myeloid leukemia. In fact, the absolute weight of the white pulp remains unchanged, indicating that it is not affected by this disease. The exclusive proliferation of the red pulp without any evidence of sinus hyperplasia pointing to a circulatory factor suggests that the undifferentiated splenic reticulum has been stimulated but without further hematic differentiation. That the hyperplastic reticulum cells, however, retain their original hemoblastic competence becomes evident in those cases of polycythemia which present final myeloid metaplasia and a leukemic blood picture.

It has been previously suggested that the growth of the trabeculae might be a function of the duration of the disease. If this is so, one would expect that a disease like polycythemia in a leukemoid phase,

7. Delannoy, E.: Un cas de maladie de Vaquez, Paris, G. Doin, 1924.

8. Klemperer, P.: The Spleen, in Downey, H.: Handbook of Hematology, New York, Paul B. Hoeber, Inc., 1938.

in which the histologic aspect is similar to that of leukemia but the clinical course is more prolonged, would show a larger percentage of trabeculae. In such a case in which we were able to make measurements, this hypothesis was confirmed. Despite enormous enlargement of the organ (3,900 Gm.), the percentage of trabeculae was 6.13 ± 1.33 ; the white pulp was completely absent; the red pulp amounted to 93.87 per cent.

Chronic Disturbance of the Circulation of the Blood.—From a consideration of the fact that splenomegaly occurs only occasionally in cardiac failure, it is apparent that increased systemic venous pressure, of itself, does not lead to enlargement of the spleen. Unfortunately, our study of the 6 cases of chronic cardiac failure did not yield percentages differing statistically from the normal. It is possible that investigation of a much larger sample might do so.

On the other hand, the group of cases of obstruction of the splenic and portal veins without cirrhosis of the liver show conspicuous splenomegaly. This has been generally regarded as the result of the stimulation of the cytoplasmic reticulum of the red pulp. It is commonly referred to as congestive splenomegaly. Moschowitz⁹ has recently pointed out that the stimulus of chronic hypertension of the portal circulation is the decisive factor in the structural transformation which the spleen undergoes in this situation. Our results show a striking diminution in percentage of white pulp, although the absolute weight remains unchanged. In other words, the increase in size is due almost exclusively to red pulp growth, the white pulp remaining untouched by the process.

The fact that the percentage of trabeculae remains relatively constant, indicating that the trabecular system keeps pace with the splenic enlargement, may reflect the response of the organ to prolonged venous hypertension or stasis. Most other forms of splenomegaly do not show this. On the other hand, the diseases of this group are usually of longer duration than the others, and it may be that trabecular growth is a slow process and bears a direct relation to the duration of the splenic enlargement.

A corollary of these results is that the reticulum apparently retains its capacity to form trabecular fibers. One might speculate that the trabecular system is continually undergoing destruction and replacement of its fibrous tissue, analogous to the changes which are thought to occur in bone.

It is not clear why similar changes should not occur in chronic cardiac failure. It can only be pointed out that there are important

9. Moschowitz, E.: The Pathogenesis of the Splenomegaly in Hypertension of the Portal Circuit (Congestive Splenomegaly), to be published.

differences between the central (cardiac) and peripheral (portal system) types of hypertension and stasis of the splenic venous system—i. e., the presence of collateral circulation in the cases of disease of the splenic or the portal vein, and the presumed differences in the height, the duration and the intermittency of the venous pressure.

Cirrhosis.—The white pulp, although probably significantly decreased, shows much less change in percentage in splenomegaly accompanying cirrhosis than in the purely obstructive splenomegalies. In fact, the absolute weight is twice normal. This suggests strongly that the portal obstruction in Laennec's cirrhosis is not the only factor responsible for the splenomegaly. Something has stimulated the white pulp as well as the red, although not to as great a degree.

The results in cirrhosis with obstruction of veins show a higher percentage of trabeculae, in keeping with the purely mechanical factor of back pressure. If the splenomegaly were due exclusively to portal hypertension, one would expect little difference in these groups. The conclusion is probably warranted that the spleen plays more than a passive role in cirrhosis. Furthermore, the hyperplasia of white pulp favors the hypothesis that an additional factor (perhaps inflammatory) is concerned.

One of the original hypotheses of this investigation was that if the spleen were studied in a large enough group of cirrheses, some factors might emerge which would permit a subdivision of this relatively heterogeneous group of diseases in terms of the behavior of the spleen; i. e., that an analysis of the nature of the splenomegaly might lead to a better understanding of the different stages and subdivisions of the cirrheses. For example—it is probable that splenic enlargement occurs in Laennec's cirrhosis prior to the development of portal hypertension. Would a comparison of this type of spleen and one representing an advanced case of the disease reveal any important differences? Unfortunately, the number of cases available for study was insufficient to permit such an analysis.

Infectious Disease—Subacute Bacterial Endocarditis.—It has been noted⁸ that the malpighian corpuscles are often very large in this condition. We have been able to confirm that there is a decided growth of the lymphatic tissue. The figure of 8.5 per cent for the white pulp is the highest of all the results for the abnormal spleens. In pathogenetic terms, both the white and the red pulp appear to be implicated in this disease. The increase of white pulp is very likely a reflection of the role of the lymphatic tissue in the formation of antibody. One could speculate that the lymphatic percentage should be even higher in cases in which subacute bacterial endocarditis has reached the bacteria-free stage, in which presumably the body has developed a high resistance

to the invading organism. In the single case in which we were able to make measurements, however, this was not true (weight of spleen, 800 Gm.; trabeculae, 1.99 per cent \pm 0.69; white pulp, 3.38 per cent \pm 1:39; red pulp, 94.63 per cent).

In subacute bacterial endocarditis the trabeculae are decreased—presumably because of the relatively rapid evolution of the splenomegaly.

FINAL SUMMARY

A method is described for the quantitative estimation of the percentages of red pulp, white pulp and trabeculae of the spleen. The results for 30 "normal" spleens are compared with those obtained in 71 cases of splenomegaly classified pathogenetically. The different components are found to grow in different proportions in the various diseases. From these results certain inferences are drawn regarding the mechanism of splenic enlargement and the nature of the disease processes investigated.

VIRAL VERSUS TOXIC HEPATIC NECROSIS

HANS POPPER, M.D., Ph.D.
AND
MURRAY FRANKLIN, M.D.
CHICAGO

THAT acute hepatitis may be due to a hepatotoxic virus has been demonstrated by epidemiologic investigations and studies on human volunteers.¹ The fatal and the benign form of this condition were previously called acute yellow atrophy and catarrhal jaundice, respectively. Epidemiologic considerations were primarily responsible for the acceptance of a viral causation of the different forms of hepatitis encountered in the armed forces of almost all nations engaged in World War II. However, the question arises whether in the civilian population most instances of a similar clinical picture of primary (benign or fatal) acute hepatitis without known epidemiologic background are of the same, presumably viral, causation. One method of approach to the solution of this problem is a pathologic analysis of the cases of primary fatal hepatic necrosis (commonly called hepatitis) observed in a large civilian general hospital during the past eighteen years and a comparison of the morphologic picture with that of similar cases observed in military personnel. All instances in which the hepatic damage was a complication of another disease causing death were omitted from this study. Excellent studies² from the Army Institute of Pathology on the fatal form of this acute hepatic necrosis (epidemic hepatitis) in military personnel serve as the basis for such a comparison. The question of the causation of primary hepatitis in civilians is significant from the diagnostic, the prognostic and the therapeutic standpoint.

The literature on this point is not too clear. Epidemic outbreaks of hepatitis occurring in wartime, especially in military personnel, have

From the Hektoen Institute for Medical Research and the Departments of Pathology of Cook County Hospital and Northwestern University Medical School.

This investigation was supported by a grant from the Committee on Scientific Research of the American Medical Association and from the Dr. Jerome D. Solomon Memorial Research Foundation.

1. (a) Neefe, J. R.: *M. Clin. North America* **30**:1407, 1946. (b) Havens, W. P., Jr.: *J.A.M.A.* **134**:653, 1947. (c) Oliphant, J. W., in *Harvey Lectures*, Baltimore, Williams & Wilkins Company, 1943, vol. 39, p. 254.

2. (a) Lucké, F.: *Am. J. Path.* **20**:471, 1944. (b) Lucké, B., and Mallory T.: *ibid.* **22**:867, 1946.

been known for a good many years.^{2a} Epidemics of this disease occurring in the civilian population in peacetime have been repeatedly reported from various countries.³ It was only after a series of outbreaks in the second World War that the viral causation of epidemic hepatitis was fully accepted.⁴ Previously, bacteria, especially those of the enteric group, had been considered the infectious etiologic agents by many.⁵ The viral causation of epidemic hepatitis was indicated not by actual culture but by successful transmission to human volunteers of a material which is temperature resistant and is not destroyed by low concentrations of disinfectant.¹ In contrast to the naturally occurring hepatitis, transmitted by the gastrointestinal route, the form known as homologous serum jaundice is due to the parenteral administration of infected blood products via blood and plasma transfusion, vaccinations and use of improperly sterilized syringes.⁶ The naturally occurring infectious hepatitis seems to differ immunologically from, and has a much shorter incubation period than, homologous serum jaundice.

After the exclusion of obvious cases of toxic hepatitis due to known hepatotoxic agents, the question arises whether the remaining instances of sporadic hepatitis are all viral in origin. Some investigators are willing to make this assumption,⁷ while others⁸ consider epidemic and sporadic catarrhal jaundice different diseases. Eppinger's⁹ monograph

3. (a) Frohlich, C.: *Deutsches Arch. f. klin. Med.* **24**:394, 1879. (b) Cockayne, E. A.: *Quart. J. Med.* **6**:1, 1912. (c) Symmers, D.: *J.A.M.A.* **74**:1153, 1920. (d) Jones, C. M., and Minot, G.: *Boston M. & S. J.* **189**:531, 1923. (e) Blumer, G.: *J.A.M.A.* **81**:353, 1923. (f) Chomet, B.: *Med. Klin.* **30**:1428, 1934. (g) Bohrmann, U. F.: *Ergebn. d. inn. Med. u. Kinderh.* **58**:201, 1940. (h) Hardy, L. H., and Feemster, R.: *New England J. Med.* **235**:147, 1946. (i) Wallgren, A.: *Acta paediat.* **9** (supp. 2):1, 1930. (j) Wickstrom, J.: *ibid.* **28**:385, 1940. (k) Alsted, G.: *Am. J. M. Sc.* **213**:257, 1947. (l) Bergstrand, H.: *Ueber die akute und chronische gelbe Leber Atrophie*, Leipzig, G. Thieme, 1930.

4. (a) Cameron, J. D. S.: *Quart. J. Med.* **12**:139, 1943. (b) MacCallum, F. O.: *Brit. M. Bull.* **1**:112, 1943. (c) Demadarin, K., and Harfall, J. T.: *Brit. M. J.* **2**:587, 1944. (d) Gezon, H. M.: *U. S. Nav. M. Bull.* **43**:579, 1944. (e) Barker, M. H.; Capps, R. B., and Allen, F. W.: *J.A.M.A.* **128**:997, 1945. (f) Gutzeit, K.: *München. med. Wchnschr.* **89**:161, 1942. (g) Dietrich, S.: *Deutsche med. Wchnschr.* **68**:5, 1942. (h) Holler, F.: *ibid.* **68**:724, 1942.

5. (a) Ottenberg, R., and Spiegel, R.: *Medicine* **22**:27, 1943. (b) Lichtman, S. S.: *Diseases of the Liver, Gall Bladder and Bile Ducts*, Philadelphia, Lea & Febiger, 1942.

6. Morgan, H. V., and Williamson, D. A. J.: *Brit. M. J.* **1**:750, 1943. Beeson, P. D.: *J.A.M.A.* **121**:1332, 1943. Findley, G. M.; Martin, N. H., and Mitchell, J. B.: *Lancet* **2**:301 and 365, 1944. Sawyer, W. A.; Meyer, K. F.; Eaton, M. D.; Bauer, J. H.; Putnam, P., and Schwentker, F. F.: *Am. J. Hyg.* **39**:337, 1944; **40**:35, 1944. Bigger, J. W.: *Lancet* **1**:457, 1943. Neefe.^{1a} Havens.^{1b}

7. Bohrmann.^{3g} Wallgren.³¹

8. Selander, P.: *Acta paediat.* **23** (supp. 4):1, 1939.

9. Eppinger, H.: *Die Leberkrankheiten*, Berlin, Julius Springer, 1937.

fails to mention the epidemic nature of catarrhal jaundice, and one of his co-workers¹⁰ even attempted to disprove the possibility of its infectious causation. An early attempt at differentiating between epidemic catarrhal jaundice and other forms of contagious and noncontagious jaundice was made¹¹ but was difficult due to lack of etiologic knowledge.

The histologic picture of primary hepatitis has been thoroughly studied. The picture of the benign form as first described by Eppinger⁹ was based on the study of livers of soldiers suffering from this disease who had died a traumatic death. He concluded that catarrhal jaundice is the nonfatal form of acute atrophy of the liver and called the disease parenchymal hepatitis. His findings were confirmed by the study of biopsy and necropsy specimens.¹² In recent years the widely expanded use of the hepatic biopsy has permitted a thorough study of the pathologic aspects of the nonfatal type.¹³

Fatal hepatic necrosis is a subject of extensive description in the textbooks of pathology, not always, however, with clear differentiation of the different forms. The recent wave of fatal epidemic hepatitis is reflected in a large series of anatomic descriptions of the disease from military personnel¹⁴ and civilians.¹⁵ The most thorough studies have been performed by Lucké and Mallory,² at the Army Institute of Pathology. There are ample descriptions of the hepatic necrosis occurring in man as a result of the action of known toxins¹⁶—for instance,

10. Lainer, F.: *Wien. klin. Wchnschr.* **53**:601, 1940.

11. Wilcox, W. H.: *Brit. M. J.* **1**:565, 605 and 639, 1919.

12. Wessel, C.: *Acta path. et microbiol. Scandinav.* **58**:533, 1924. Nordmann, O.: *Med. Klin.* **21**:1746, 1925. Klemperer, P.; Killian, J. A., and Heyd, C. G.: *Arch. Path.* **2**:631, 1926. Schrumph, A.: *Ann. d'anat. path.* **9**:17, 1932. Gaskell, J. F.: *J. Path. & Bact.* **36**:257, 1933. Barber, H., and Osborn, G. R.: *ibid.* **49**:581, 1934. Popper, H.: *Wien. klin. Wchnschr.* **49**:207, 1936.

13. (a) Iversen, P., and Roholm, K.: *Acta med. Scandinav.* **102**:1, 1939. (b) Roholm, K., and Iversen, P.: *Acta path. et microbiol. Scandinav.* **16**:427, 1939. (c) Dible, J. H.; McMichael, J., and Sherlock, S. P. V.: *Lancet* **2**:402, 1943. (d) Axenfeld, H., and Brass, K.: *Frankfurt. Ztschr. f. Path.* **57**:147, 1942; **58**:220, 1944; **59**:281, 1948. (e) Mallory, T. B.: *J. A. M. A.* **134**:655, 1947. (f) Rappaport, E., and Klatskin, G.: *Rev. Gastroenterol.* **14**:17, 1947.

14. Wood, D. A.: *Arch. Path.* **41**:345, 1946. Siegmund, H.: *Virchows Arch. f. path. Anat.* **311**:180, 1944. Berk, J. E.: *Gastroenterology* **8**:296, 1947. Stokes, J. F., and Miller, A. A.: *Quart. J. Med.* **16**:211, 1947. Cameron.^{4a}

15. Taylor, H. E.: *Am. J. Clin. Path.* **17**:314, 1947. Fox, J. P.; Manso, C.; Penna, H. A., and Madureira, P.: *Am. J. Hyg.* **36**:68, 1942. Nicod, J. L.: *Gastroenterologia* **71**:62, 1946. Roulet, F.: *Virchows Arch. f. path. Anat.* **310**:436, 1943. Wood, D. A., and Black, M. B.: *Am. J. Clin. Path.* **16**:746, 1946.

16. Hanser, R.: *Atrophie, Nekrose, Ablagerungen und Speicherungen (sog. Degenerationen)*, in Henke, F., and Lubarsch, O.: *Handbuch der speziellen pathologischen Anatomie und Histologie*, Berlin, Julius Springer, 1930, vol. 5, pt. 1.

carbon tetrachloride,¹⁷ chloroform,¹⁸ phosphorus,¹⁹ mushroom poison²⁰ and the toxins developing after severe burns.²¹ In the older literature, however, emphasis had already been focused on the difference between the hepatic necrosis due to known poisons, such as phosphorus, and the so-called yellow atrophy of unknown cause.²²

MATERIAL AND METHOD

The material of this study includes 136 patients with fatal primary hepatic necrosis who came to autopsy at the Cook County Hospital during the period of 1929 through 1947. The total number of autopsies in this period was 23,028. As already mentioned, only those patients were included who had jaundice and in whom hepatic insufficiency was the cause of death. Clinically they were considered as having hepatitis. Their hepatitis was not a complication of another disease which was in the foreground of the picture. Anatomically, these cases of disease of the liver have been recorded as cases of acute or subacute atrophy of the liver or as cases of fatal hepatitis. Cases of acute damage of the liver in cirrhosis were not incorporated. It was often difficult to draw a line between cirrhosis and chronic hepatitis. In general, cases in which there was far advanced distorted reconstruction of the lobular pattern of the liver were excluded. The instances of fatal chronic hepatitis included actually represent examples of the larger group of postnecrotic cirrhosis. Histologic material was available in 95 cases, and in some of these the gross specimens could be studied.

The material from military personnel used for comparison was obtained from two Army hospitals which served during the war as histopathologic centers. It is not consecutive in character and permits, therefore, no statistical evaluation as to incidence.

The liver tissue was in most instances fixed in 10 per cent formaldehyde solution U.S.P. Occasionally, material preserved in acetic acid-Zenker solution, formaldehyde-Zenker solution or in Carnoy solution was available. In all cases, sections stained with hematoxylin and eosin were studied. They were usually supplemented with sections stained with Mallory's aniline blue and Gömöri's or the Foot-Bielschowsky reticulum fiber stain.

RESULTS OF STUDIES ON MATERIAL FROM MILITARY PERSONNEL

The livers of 15 among 18 soldiers dying of primary hepatic necrosis presented a morphologic picture similar to that of fatal epidemic hepatitis as described by Lucké and Mallory.² These cases were classified as a "viral" group.

I. VIRAL GROUP

In this group, in which the viral causation is strongly suggested by the epidemiologic background of Army life and exposure, examples

17. Peery, T. M.: *Arch. Path.* **26**:923, 1941.

18. Fischler, F.: *Mitt. a. d. Grenzgeb. d. Med. u. Chir.* **26**:553, 1913.

19. LaDue, T. S.; Schenken, J. R., and Kuker, L. H.: *Am. J. M. Sc.* **208**:223, 1944.

20. Klemperer, P.: *Virchows Arch. f. path. Anat.* **237**:400, 1922.

21. Hartman, F. W., and Romence, H. L.: *Ann. Surg.* **118**:402, 1943.

22. Paltauf, R.: *Verhandl. d. deutsch. path. Gesellsch.* **5**:91, 1902. Klemperer.²⁰

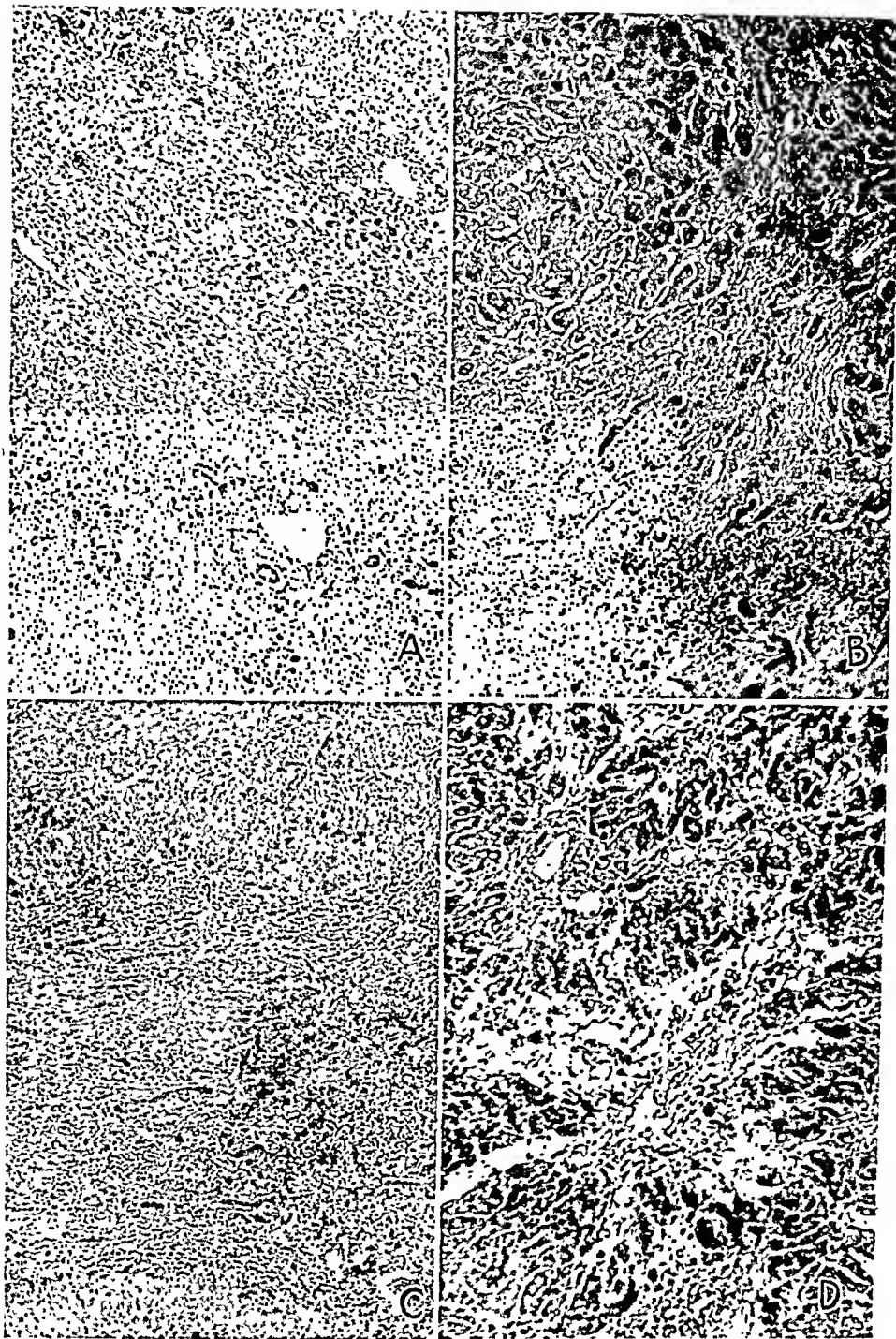


Fig. 1.—*A*, fulminant form of viral necrosis with almost complete disappearance of liver cells, in a soldier. In the meshes of the denuded framework are phagocytic mononuclear cells; such cells accumulate also in the periportal field.

B, subacute form of viral necrosis in a soldier. In the greater part of the section the connective tissue framework is denuded of liver cell cords and replaced by mononuclear exudate cells and a few regenerating liver cell cords resembling bile ducts. In addition, nodules composed of liver cell cords, not arranged around a central vein, are seen.

C, chronic viral necrosis in a soldier. Irregularly arranged nodules which have resulted from distorted reconstruction are separated by wide bands in which many mononuclear cells and liver cell cords resembling bile ducts are found.

D, central toxic necrosis in a soldier. The central half of the nodule is denuded of liver cells. In the meshes of the collapsed framework are a few exudate and Kupffer cells. The periportal fields are free of exudate cells.

of the acute fulminating (fig. 1 *A*), subacute (fig. 1 *B*) and chronic (fig. 1 *C*) forms were found, as summarized in table 1. The clinical histories of the patients, the laboratory findings and the gross and histologic pictures follow the classic description of Lucké and Mallory.² A detailed analysis would be repetitious. In the acute form, rapid disintegration of the liver cells and marked mesenchymal reaction are in the foreground.

In most of the studied cases the destruction was acute in character, with little evidence of regeneration. The hepatitis was fulminant in accordance with the nomenclature of the Army Institute of Pathology. As expected, in military material, the third decade of life, the male sex and the white race are predominant. In more than half of the cases there were recognized possible etiologic factors, such as plasma or

TABLE 1.—*Studied Military Cases of Fatal Hepatic Necrosis*

Stage or Form	Cases	Average Days Since Outbreak of Jaundice	Average Age	Sex		Race		Etiologic Factors
				Male	Fe- male	White	Negro	
Cases of "Viral" Type								
Stage:								
Fulminant.....	9	5.9	26.9	9	0	8	1	Blood transfusions.. 3
Subacute.....	5	25.0	31.8	4	1	4	1	Plasma transfusions 2
Chronic.....	1	165.0	21.0	1	0	0	1	Arsenical injections.. 1
								War injuries..... 2
Totals.....	15		28.1	14	1	12	3	Unknown..... 7
Cases of "Toxic" Type								
Form:								
Central necrosis.....	3	9	30	3	0	2	1	Known 8
								Unknown 7
								Bout of acute alco- 1
								hollism

blood transfusions. In 1 instance, intravenous injections of arsenical compounds was recorded, and from the time elapsed between the injection and the appearance of jaundice, a diagnosis of homologous serum hepatitis was possible.

II. TOXIC GROUP

Among the studied cases of fatal hepatitis in Army personnel, 3 did not conform with the given description of viral hepatitis, although clinically they were also characterized by hepatic insufficiency and morphologically by far reaching destruction of the parenchyma of the liver. One case is presented as an example:

A 44 year old white soldier had had an attack of jaundice thirty years before death while he was in Central America. Ten years later he had malaria. About ten years before he was last admitted to the hospital he received 106 injections of arsenical and bismuth compounds for syphilis. He also stated that he had drunk ½ to 1 pint (about 300 cc.) of whisky daily for the past twenty years. Twenty-four

hours after a bout of heavy drinking, he acquired a fever of 102.4 F., and had pain in the right upper quadrant of the abdomen and diarrhea and started vomiting. He became moderately jaundiced, and the liver was palpable 3 fingerbreadths below the costal margin. The spleen was not palpable. There was tenderness in the right subcostal region. The urine contained bilirubin, a trace of sugar and albumin but no leucine or tyrosine crystals. The red blood cell count was 4,400,000; the hemoglobin content, 90 per cent. The white blood cell count was 9,800, of which 80 per cent were polymorphonuclears, 18 per cent lymphocytes and 2 per cent monocytes. The icterus index was 80; the immediate direct van den Bergh reaction was positive; the serum nonprotein nitrogen was 160 mg. per hundred cubic centimeters; the serum albumin was 4.2 Gm. and the serum globulin 3.7 Gm. per hundred cubic centimeters. The jaundice gradually deepened, and slowly anuria developed. The patient died eight days after the onset of the jaundice in uremia.

At autopsy the liver weighed 1,860 Gm. Its surface was moist and the capsule thickened. The organ was slightly firmer than normal. The cut surface was brownish yellow and mottled with small depressed areas. Microscopically (fig. 1A), in the central and intermediate zone of the lobules the connective tissue framework appeared collapsed and almost denuded of liver cells. The reticulum fibers as such were not interrupted. The Kupffer cells were moderately proliferated and laden with biliary material. Few histiocytic phagocytes were present. Red cells accumulated in and around the sinusoids. In the border zone of these areas, remnants of liver cells were seen without nuclear staining. They were eosinophilic and revealed shadows of the cellular structure. Around them were large cell fragments of similar appearance. There were almost no inflammatory changes. The liver cells in the periphery of the lobules appeared intact and contained small and large fat droplets. The cords were separated from the wall of the sinusoids by edema fluid. The periportal fields were small and devoid of cellular reaction. A few inflammatory cells were found in the walls of the central veins.

Comment.—In age and sex distribution these 3 cases are identical with those of the previous group (table 1). The interval of time between the outbreak of jaundice and death is apparently longer in this than in the fulminant viral group, although the series is too small for a definite conclusion. Histologically, the lesion is definitely zonal, and in the 3 examined cases restricted to the central portion of the lobule. The liver cells in the peripheral part appear intact. However, they are swollen and surrounded by edema fluid. On the border between the intact and the necrotic area a gradual transition is noted in that the liver cells may reveal small fat droplets or small or large, bright red, strongly refractile, irregularly shaped clumps as a result of coagulation necrosis. They are often arranged around the nucleus and show a tendency to coalesce. Finally, the nuclei do not stain and may disintegrate, and in the central areas large remnants and fragments of liver cells are observed. Most of them are anuclear, but the cellular structure is still present in a ghostlike fashion, even though part of the cytoplasm appears coagulated. Small phagocytosed cell fragments are rare. The reticular framework is intact but collapsed, and in its meshes, in addition to the liver cell fragments, a few exudate cells, some of

them polymorphonuclear in type, may be seen. There are few exudate cells in the periportal field, and cords of regenerating liver cells are not conspicuous. Bile duct proliferation is almost entirely absent.

COMPARISON OF THE TWO GROUPS

The first group discussed, which appears identical with the cases described by Lucké and Mallory,² is assumed to be cases of the so-called infectious or epidemic hepatitis. The etiologic agents in this group are, according to our present knowledge, the viruses responsible for epidemic and homologous serum hepatitis. This "viral" group is characterized by rapid disintegration of liver cells with formation of chiefly small cell fragments hardly recognized in routine stains, denudation of the framework with marked mesenchymal cellular reaction and phagocytosis, involvement of the entire lobule and cords of regenerating liver cells which simulate bile ducts in the lobular periphery.

The second form is characterized by predominantly zonal involvement, with gradual death of the liver cells. The diseased cells reveal fatty metamorphosis and coagulation necrosis. However, the cell remnants or large cell fragments in which the nuclei are not stained any longer or have disintegrated still permit recognition of cellular structure (ghost cells). The framework is denuded without marked inflammatory reaction, and few regenerative changes are noted. In 1 instance in this group an acute alcoholic bout was elicited as the etiologic factor.

The morphologic differences between the first and the second group lead to the assumption of a different causation and, therefore, also a different designation for the second group. Only in 1 instance was an actual hepatotoxic etiologic factor elicited. Nevertheless, the morphologic picture is similar to that seen in patients as a result of the action of known hepatotoxic poisons, such as carbon tetrachloride, or that of the central necrosis so well described by F. B. Mallory²³ many years ago, which is found as a common complication although usually not as a cause of death in many conditions in which toxic factors, such as bacterial, endogenous or exogenous poisons, are established. Since the difference between such pictures and the one in our cases of this group is a matter of degree, the designation "toxic necrosis" or "toxic hepatitis" appears justified. A similar nomenclature had already been applied to such cases several years ago.²⁴

RESULTS OF STUDIES OF MATERIAL FROM CIVILIANS

Over the period studied (1929 to 1947) the 136 cases of primary fatal necrosis of the liver represent an average of 0.59 per cent of the entire autopsy material. Although in recent years this percentage

23. Mallory, F. B.: *J. M. Research* 6:264, 1901.

24. Kirschbaum, J. D., and Popper, H.: *Arch. Int. Med.* 65:465, 1940.

has been somewhat exceeded,²⁵ yet in general the distribution over the years remained fairly constant (fig. 2). In this material 47 per cent of the patients were male and 53 per cent female; 58 per cent were white and 42 per cent Negro; the age ranged from 3 weeks to 78 years. A possible etiologic factor was recorded in almost one-third of the cases (table 2). Most often mentioned was administration of arsenical compounds; next came acute alcoholic bout and pregnancy. Furthermore, in the material studied there were a number of accompanying conditions (table 3) which, although not the cause, may have had a modifying influence on the changes in the liver.

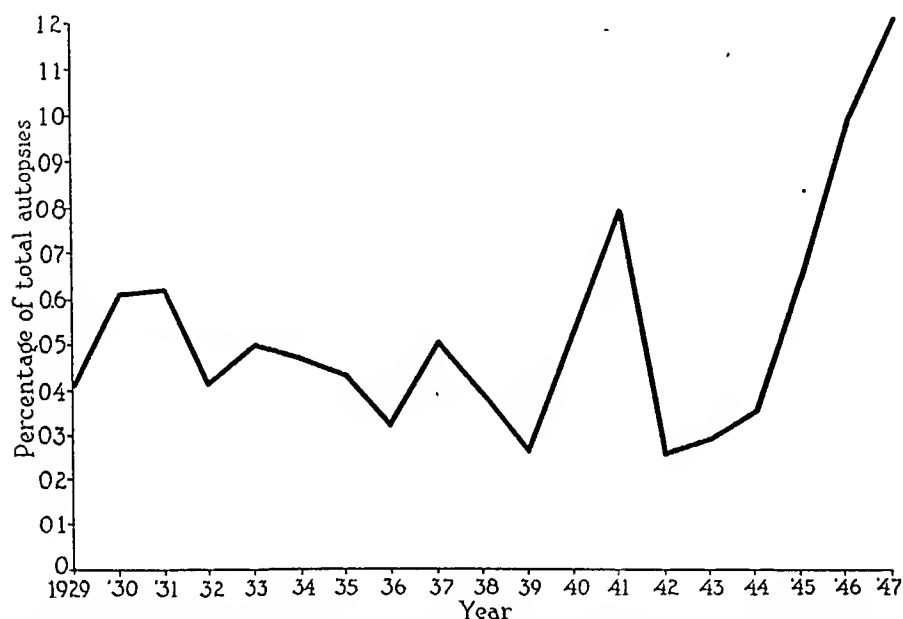


Fig. 2.—Yearly incidence of acute fatal hepatic necrosis in autopsies at Cook County Hospital, Chicago.

MORPHOLOGIC EXAMINATION

The morphologic criteria used in military personnel for differentiating between a viral and a toxic group were applied to the 95 civilian cases in which histologic material was available. In 26 of them the picture resembled that seen in the cases classified as viral in the Army material, whereas the remaining 69 cases were tentatively classified as instances of toxic hepatitis.

I. VIRAL GROUP

Following the classification used with the military material, the cases of the viral type were subdivided into those of a fulminant, those

25. Reference deleted by the authors.

TABLE 2.—Possible Etiologic Factors in 136 Cases of Fatal Acute Hepatic Necrosis

Etiologic Factors	Cases	Percentage of Total
Administration of arsenical compounds.....	10	7.4
Blood transfusions.....	7	5.18
Acute alcoholism.....	4	2.96
Pregnancy	4	2.96
Administration of mercurial compounds.....	3	2.24
Administration of sulfonamide compounds.....	3	2.24
Contact with patients having catarrhal jaundice.....	2	1.48
Administration of barbiturates.....	2	1.48
Administration of cinchophen.....	1	0.74
Lead	1	0.74
Attempted suicide with "creolin".....	1	0.74
Turpentine	1	0.74
Salmonella paratyphi.....	1	0.74
Total.....	40	29.8

TABLE 3.—Conditions Accompanying Fatal Acute Hepatic Necrosis in 136 Cases

	Cases
Syphilis.....	14
Tuberculosis.....	3
Chronic alcoholism.....	7
Diabetes mellitus.....	2
Malnutrition and avitaminosis.....	3
Trauma.....	3
Arthritis.....	1
Scarlet fever.....	1
Staphylococcus infection.....	1
Epilepsy.....	1
Pregnancy (complicating arsenical hepatitis).....	1
Thrombopenic purpura.....	1
Total.....	38

TABLE 4.—Studied Civilian Cases of Fatal Hepatic Necrosis

Stage or Form	Cases	Average Days Since Outbreak of Jaundice	Average Age	Sex		Race		Etiologic Factors
				Male	Fe- male	White	Negro	
Cases of "Viral" Type								
Stage:								
Fulminant.....	12	6.1	34.3	5	7	7	5	Blood transfusions.. 4
Subacute.....	9	23.5	35.7	3	6	6	4	Injection of arsenical compounds 2
Chronic.....	5	42.0	41.7	3	2	2	4	Contact with "catarrhal" jaundice 1
Totals.....	26	—	36.2	11	15	15	11	Known 7 Unknown 19
Cases of "Toxic" Type								
Form:								
Central necrosis.....	44	10.4	43.0	20	24	25	19	Administration of: Mercurial compounds 1
								"Creolin"..... 1
Fatty necrosis.....	15	8.5	40.1	6	9	8	7	Trinitrotoluene..... 1
								Arsenical compounds 2
Peripheral necrosis..	3	17.5	38.0	1	2	2	1	Barbiturates..... 2
Transition into post-necrotic cirrhosis	7	39.2	46.2	4	3	5	2	Carbon tetrachloride 2
								Sulfonamide compounds 2
								Alcoholism..... 2
Total.....	69	—	42.5	31	38	40	29	Known 13 Unknown 56

of a subacute and those of a chronic stage (table 4), for each of which an example is presented.

Fulminant "Viral" Hepatitis.—The patient was a 20 year old Negro girl whose medical and surgical history was essentially negative and whose menstrual history was normal. Four days before she was admitted to the hospital, moderately severe intermittent epigastric pain developed, radiating to her back. The following day a local physician noticed jaundice and advised that she rest in bed. She became increasingly drowsy and comatose. On admission her temperature was 101.8 F. Hepatic dulness was absent on percussion over the right lower two ribs anteriorly. There was bilateral sustained ankle clonus. The urine contained some albumin and considerable bilirubin. The blood dextrose was 41 mg. per hundred cubic centimeters; the carbon dioxide-combining power, 22 per cent by volume; the serum nonprotein nitrogen was 61 mg. and creatinine 2.2 mg. per hundred cubic centimeters; the icteric index was 60. Despite intravenous administration of dextrose, saline solution, plasma, Ringer's solution, lactated, U.S.P., and penicillin, she grew steadily worse and died fifteen hours after admission.

At autopsy the flabby, brownish red liver weighed 1,090 Gm. The wrinkled capsule was thin and smooth; in the cut surface lobular markings were not made out in the greater part of the organ, which here revealed a deep red color. Only in circumscribed foci were the markings recognized, the acinous centers being dark red and the peripheral areas light yellow. Histologically (fig. 3A), the connective tissue framework was completely denuded of liver cells except for a small rim on the periphery of the lobule. The intermediate and peripheral zone showed an extremely dense infiltration with round-cellular elements, many of them macrophages. The central areas showed only a moderate infiltration of these elements. Their abundant cytoplasm was often laden with bile-stained material and occasionally contained a few small fat droplets. Between them small, diffusely eosinophilic cell fragments were seen in large number. A few of the liver cells revealed diffuse coagulation necrosis with loss of nuclear staining and cellular detail; they resembled Councilman bodies because of their hyaline, refractile appearance. The Kupffer cells were proliferated, phagocytic and hardly differentiated from the macrophages. Lymphocytes, plasma cells and eosinophils were seen in moderate amount. Neutrophilic granulocytes were almost absent. In the periphery of the lobules many regenerating liver cells in short cords, often revealing fatty changes, were found. The dilated bile capillaries frequently contained exudate cells or bile thrombi. The shape of the nuclei differentiated the cords from proliferated bile ducts.

Subacute "Viral" Hepatitis.—A 13 year old Negro girl was perfectly well until three months prior to being admitted to the hospital, when intermittent epigastric pain and severe headaches developed. One month later she became icteric and her urine turned dark. Two weeks later her abdomen swelled and edema of the ankles and feet developed. She felt weak and was dyspneic on exertion. She became disoriented and began to vomit. Her temperature was 100 F. She had hyperactive reflexes; the liver, the spleen and the lymph nodes were not palpable. The otherwise normal urine contained bile. The red and white blood cell counts were normal; the serum nonprotein nitrogen was 25 mg. per hundred cubic centimeters; the total serum protein was 6.7 Gm. per hundred cubic centimeters; the prothrombin time was normal; the cephalin-cholesterol flocculation was 3 plus; the spinal fluid was normal. She died on the seventh hospital day in cholemia.

At autopsy, the greenish brown liver weighed 850 Gm., and its capsule was smooth. The surface revealed reddish irregular mottling, especially marked over the right lobe. The consistency was markedly diminished. The brownish green cut

surface showed reddish mottling throughout, with the acinous centers being made out only in places. Quite often were seen yellow nodules, up to 4 mm. in diameter, which showed no lobular pattern. The microscopic picture (fig. 3B) revealed great polymorphism. In some areas the connective tissue framework of the lobules was collapsed, with almost complete disappearance of the liver cells. The vascular pattern, however, was well preserved, and some irregularly proliferating strands

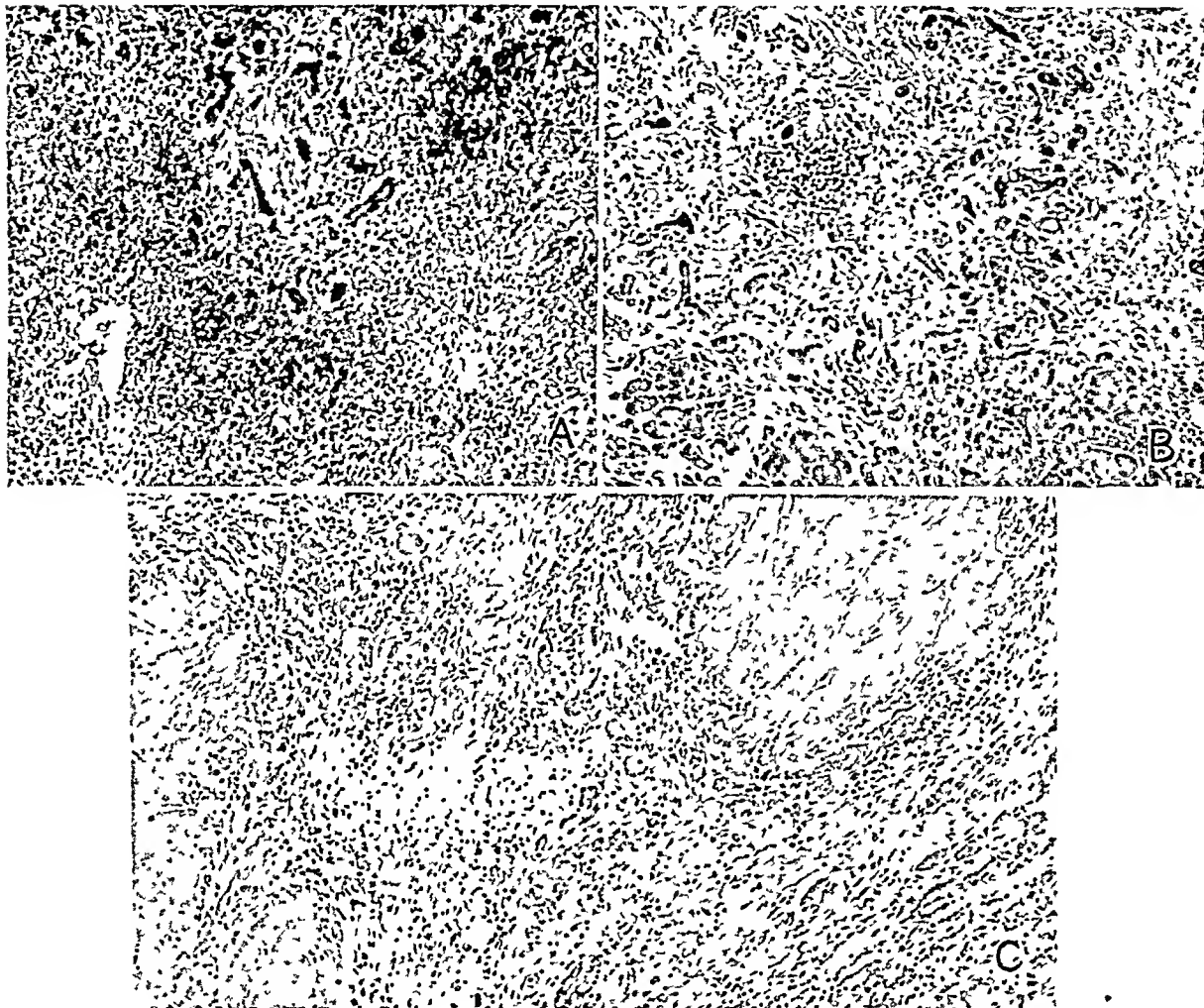


Fig 3—*A*, fulminant form of viral necrosis in a civilian. In the meshes of the denuded connective tissue framework are many red cells and phagocytic mononuclear cells. The latter also infiltrate densely the portal triads. A few irregular cords of regenerating liver cells may be noted.

B, subacute form of viral necrosis in a civilian. Areas of collapsed connective tissue reveal mononuclear exudate cells and irregularly regenerating liver cell cords. These surround small nodules composed of liver cell cords without normal lobular pattern.

C, chronic form of viral necrosis (postnecrotic cirrhosis) in a civilian. Irregularly arranged pseudolobules are surrounded by wide bands of collapsed framework in which there are round exudate cells and regenerating liver cell cords resembling septal bile ducts.

of liver cell cords were noted. They often showed formation of multiple files of liver cell cords and dilated central bile capillaries. Between the cords were many round phagocytic exudate cells; hardly any liver cell fragments were seen. The dilated sinusoids, engorged with red cells, were often of almost angiomatous character. In a few areas, however, normal liver cell cords were seen, partially arranged in regenerated nodules without characteristic pattern. In the centers of the lobules and nodules the liver cells were atrophic and often laden with bile pigment. No mesenchymal reaction was present. The portal triads revealed moderate round cell infiltration.

Chronic Viral Hepatitis.—A 30 year old Negro woman was well until three months prior to being admitted to the hospital, then sharp midback and abdominal pains, swelling of the hands and feet and progressive jaundice gradually developed. Episodes of nausea and vomiting, as well as an increasing dyspnea, appeared later. Her symptoms gradually increased in severity. Her temperature on admission was 99 F. and her pulse rate 112. The liver and the spleen were not palpable. The urine contained considerable bile pigment but no urobilinogen. The hemoglobin content was 66 per cent; the red, white and differential blood cell counts were normal; the icterus index was 95. Despite parenteral administration of blood, fluids and vitamins, she became increasingly lethargic and died in coma.

The liver weighed 500 Gm. and was deformed. The capsule was thin and in places wrinkled. The consistency varied; the color was deep greenish red. The right lobe contained a large node, which on section consisted of greenish yellow protruding liver tissue with indistinct markings. It was surrounded by dark red tissue sinking below the cut surface in which lobular markings were fairly well made out. Circumscribed, irregularly outlined scars, free of liver tissue, contained regularly spaced vessels. Histologically (fig. 2C), the connective tissue framework of many lobules was in circumscribed areas almost completely denuded of liver cells and collapsed. However, the original vascular pattern was preserved, with the portal triads and central veins being much closer to each other than usual. The reticulum framework was not thickened or collagenized and was easily differentiated from the thick connective tissue of the portal triads. In the meshes of the framework some scattered slender cords of regenerated liver cells were noted. These simulated proliferated bile ducts but contained dilated bile capillaries and bile casts. They were surrounded by many larger and smaller phagocytic exudate cells. The portal triads themselves showed little bile duct proliferation. Round cell infiltration in and around lymphatic channels was noted. In other areas liver cell nodules predominated, which apparently were regenerated and not arranged around central veins. The nodules varied in size and were irregularly spaced. Their structure otherwise resembled the few areas in which the original lobular pattern was preserved. In both lobules and nodules central atrophy and degeneration of liver cells were occasionally seen in addition to irregularly scattered focal necroses. Areas of marked bile inhibition of Kupffer and liver cells indicated focal disturbances of bile drainage. Evidence pointing toward a protracted course of the disease were: (1) preformed liver lobules with signs that blood and bile flow had met interference; (2) collapsed areas in which destruction and inflammatory response were still present; (3) reconstructed areas.

Comment.—In general, the cases of acute hepatic necrosis in civilians selected for this group show a histologic picture which is more or less identical with that noted in the viral group in the military material.

Morphologically, the basic phenomenon is the rapid destruction of the liver cells, which in these fatal cases is rather diffuse, with some centrolobular accentuation. Gradual cell death is not found and, as Lucké^{2a} pointed out, fatty changes and coagulation necrosis are not essential parts of the picture. Large remnants or fragments of cells in which the cellular structure appears preserved, but nuclear staining is absent, are as a rule not seen. A large number of small cellular fragments, however, can be noted between the mesenchymal cells, especially in the earlier stages of the process. This becomes more conspicuous on the use of special technics, such as fluorescence microscopy.²⁶ Only rarely, in the early stages of the fulminant form, are large eosinophilic liver cell fragments seen which reveal coagulation necrosis and also some fatty metamorphosis, but, as a rule, no imbibition of bile pigment. These fragments seem, however, to disintegrate rather rapidly, and the cell structure is hardly made out. Occasionally in the acute stages, bright red, refractile, mostly round elements occur which represent diffusely hyalinized liver cells, simulating the Councilman bodies found in yellow fever. They are separated from the liver cell cords, may be surrounded by mononuclear cells and are considered characteristic for the nonfatal form of viral hepatitis.^{13e} Most of the cell fragments are phagocytosed by macrophages and proliferated and mobilized Kupffer cells. Mesenchymal macrophages in the interstitial tissue may simulate degenerated shrunken liver cells which are laden with bile pigment. In general, phagocytosis is an important part of the picture. The absence of liver cells in wide areas in which, especially in the fulminant form, the framework is denuded, is not easily recognized under low power microscopic examination, which reveals a very cellular tissue. However, as Lucké^{2a} emphasized, the cells are mostly histiocytic and macrophagic elements, chiefly of round cell type (fig. 4 A). In addition, plasma cells and lymphocytes are not uncommon. Neutrophilic polymorphonuclear leukocytes are in the background. Eosinophilic cells are sometimes prominent. A similar infiltration mostly by round cellular exudate cells is found in the portal triads, chiefly arranged around lymphatic channels. Many of the histiocytes contain pigments. Almost no fatty metamorphosis is seen except for occasional small fat droplets accumulated in some isolated regenerating cells. The cellular infiltration may be less marked in older cases. In the earlier stages regeneration takes place mostly in the form of liver cell cords, which may become wide and bizarre in character. Regenerating hepatic cell cords often simulate proliferating bile ducts but may be differentiated by the character of their nuclei and the presence of bile capillaries in the center.

The gross appearance of the liver also agrees with the description published by Lucké and Mallory.² In some of the instances of the

26. Volk, B. W., and Popper, H.: To be published.

fulminant type and in the more acute stages of the subacute type, the cut surface is diffusely dark red and sunken in and the lobular markings are absent in almost the entire liver. This appearance, caused by the disappearance of the liver cells and the collapse of the framework, simulates a hyperplastic spleen (fig. 5 *A*). More often, however, in the entire liver or in smaller or larger confluent or irregularly outlined fields which are yellow or green and prominent, the markings are distinct or even exaggerated. In addition to variations in the disappearance of liver cells, the blood content varies. The predominance of blood over cell cords, which usually is more outspoken in the central part of the lobule, produces the gross impression of passive congestion

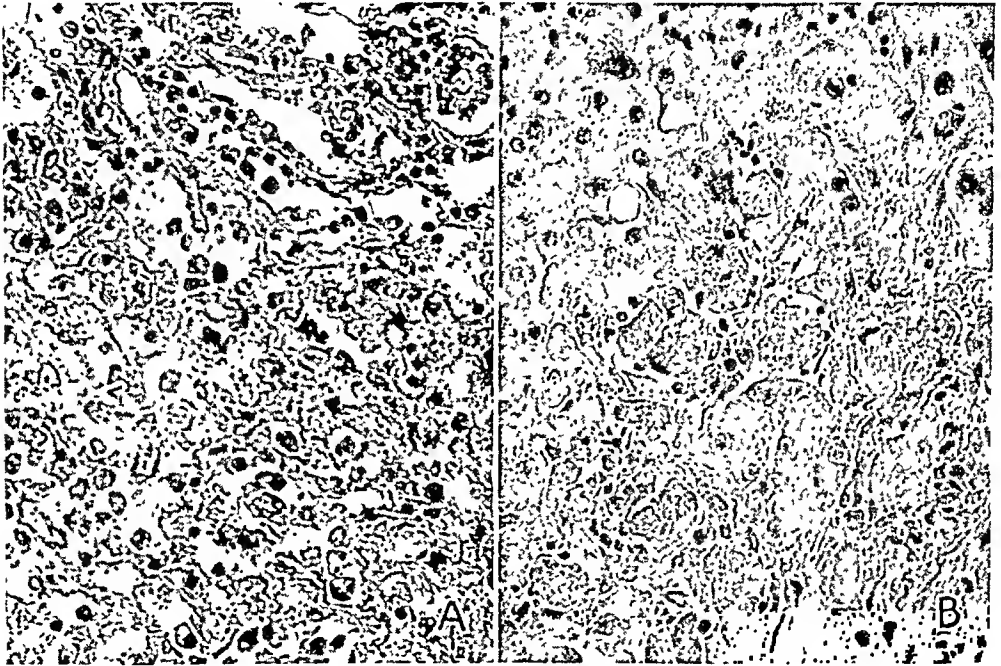


Fig. 4.—*A*, high power field of fulminant viral necrosis. No liver cells are seen. The picture is dominated by bile-laden macrophages and proliferated Kupffer cells. Between them are a few diffusely hyalinized round refractile liver cells and small hardly recognizable cell fragments. The portal triad which is indicated by a small bile duct also reveals cellular infiltration.

B, high power field of the border zone of acute central toxic necrosis. The liver cells reveal coalescing clumps of coagulated protein and some fat vacuoles. Other cells without nuclear staining reveal shadows of cellular structure, as do large liver cell fragments. Kupffer cell mobilization, but little infiltration of exudate cells, is present.

and even of an exaggerated nutmeg liver (Lucké^{2a}). There are usually great differences between the various portions of the liver, especially between the right and the left lobe (fig. 5 *B*). This variegated picture is even more outspoken on the cut surface. It is produced by different degrees of damage of the liver cells; moreover, the process seems to be of different age in different portions of the liver. In general,

the liver is flabby and collapses when placed on the autopsy table. In the subacute form of the disease, nodular foci of regeneration of different size and color give the cut section a variegated appearance, and the consistency usually does not differ much from the normal. To differentiate the chronic form from the toxic²⁷ or postnecrotic²⁸ cirrhosis is obviously difficult in view of the gradual transition from one to the other. Because of this, the selection of the material often had to be

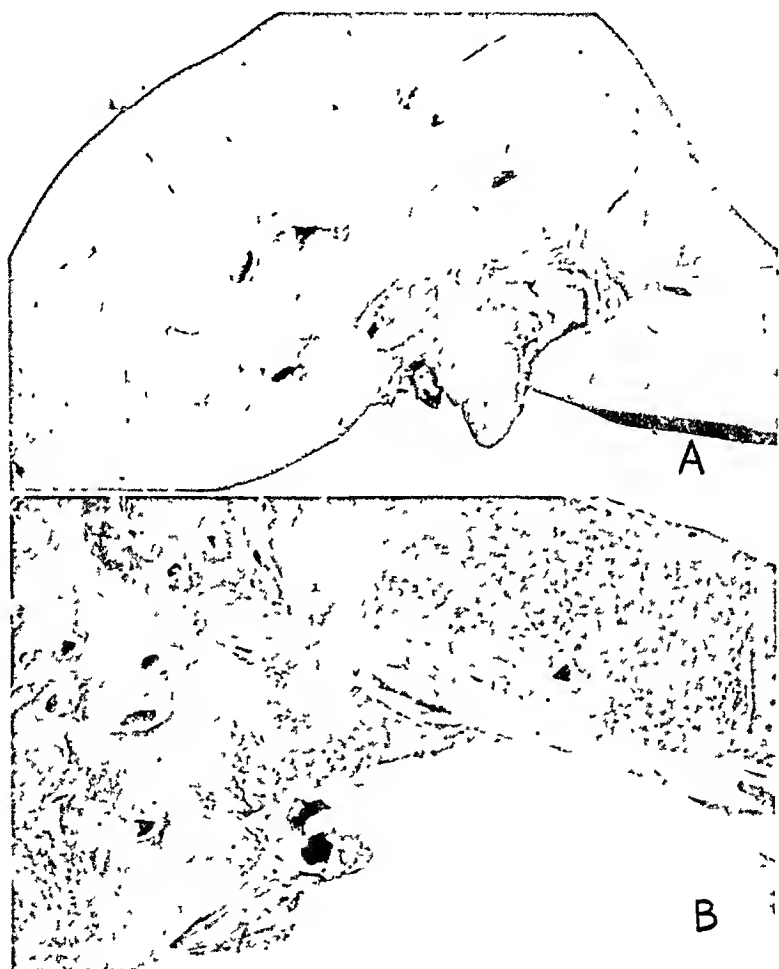


Fig. 5.—*A*, gross specimen of liver in case of acute viral necrosis. The lobular markings are entirely missing, and a spleenlike appearance is presented.

B, gross specimen showing acute viral necrosis with marked difference between various portions of the liver. Some areas have a spleenlike appearance with loss of lobular markings, whereas in other circumscribed areas (especially in the left lobe), the lobular markings are exaggerated.

arbitrary. The origin from hepatitis with necrosis of whole lobules is indicated by grossly visible scarlike bands consisting of collapsed liver

27. Mallory, F. B.: *New England J. Med.* **206**:1231, 1932.

28. Karsner, H. T.: *Am J. Clin. Path.* **13**:569, 1943.

tissue in which the closely approximated vessels are clearly apparent. Only cases in which extensive areas of the liver revealed evidence of still active viral hepatitis were included. This explains the relatively small number of cases in this group.

The average number of days between outbreak of jaundice and death is similar to that in the Army group (table 4). The age group is slightly higher, which is characteristic for the studied hospital population. In contrast to Army material, the female sex predominates. Color incidence is almost equally distributed in this group, though roughly one third of the patients admitted to the hospital were Negroes. The etiologic factors of civilian hepatitis compare with those noted in the military group. They were, however, less often elicited in view of the less accurate epidemiologic history. Moreover, in previous years exposure to blood derivatives may not have been recorded, since its significance was not known.

We can thus conclude that in civilians a picture may appear which is identical histologically with the viral hepatitis found in military personnel, and we may, therefore, be justified in considering this group as also viral in origin.

II. TOXIC GROUP

As already mentioned, in 65 civilian cases of primary fatal hepatic necrosis, the histologic picture differed from that of the previously described cases in the viral group. In the majority of these civilian cases the histologic appearance simulated those of the 3 soldiers who were placed in the toxic group with central necrosis. In addition, there were cases in which the liver revealed peripheral necrosis or predominant fatty changes. Furthermore, there were instances of chronic liver cell damage which also revealed transition into a postnecrotic cirrhosis; however, in these cases the destructive process was still acute or recurrent and followed the toxic rather than the viral pattern. Again a few cases were selected, more or less as examples of a larger group, because in them the acute destructive process was still very much in the foreground. An example of each of these four groups follows:

Acute Toxic Hepatitis with Central Necrosis.—A 42 year old white man attempted suicide by swallowing approximately 4 ounces (118 cc.) of carbon tetrachloride. He was admitted to the hospital in stuporous condition, vomiting considerable amounts of blood-tinged material. After emergency treatment consisting of gastric lavage, sedation and parenteral feeding, the patient was well until the third hospital day. Then he began to have jaundice and tenderness in the right upper quadrant of the abdomen. The following day oliguria became evident. The urine revealed at this time albumin (3 plus), bilirubin (4 plus), urobilinogen (4 plus) and an occasional red blood cell and granular cast. The serum nonprotein nitrogen was 56 mg. per hundred cubic centimeters; cephalin-cholesterol flocculation was 4 plus; thymol turbidity was 10.2 units; alkaline phosphatase was 12 Bodansky units;

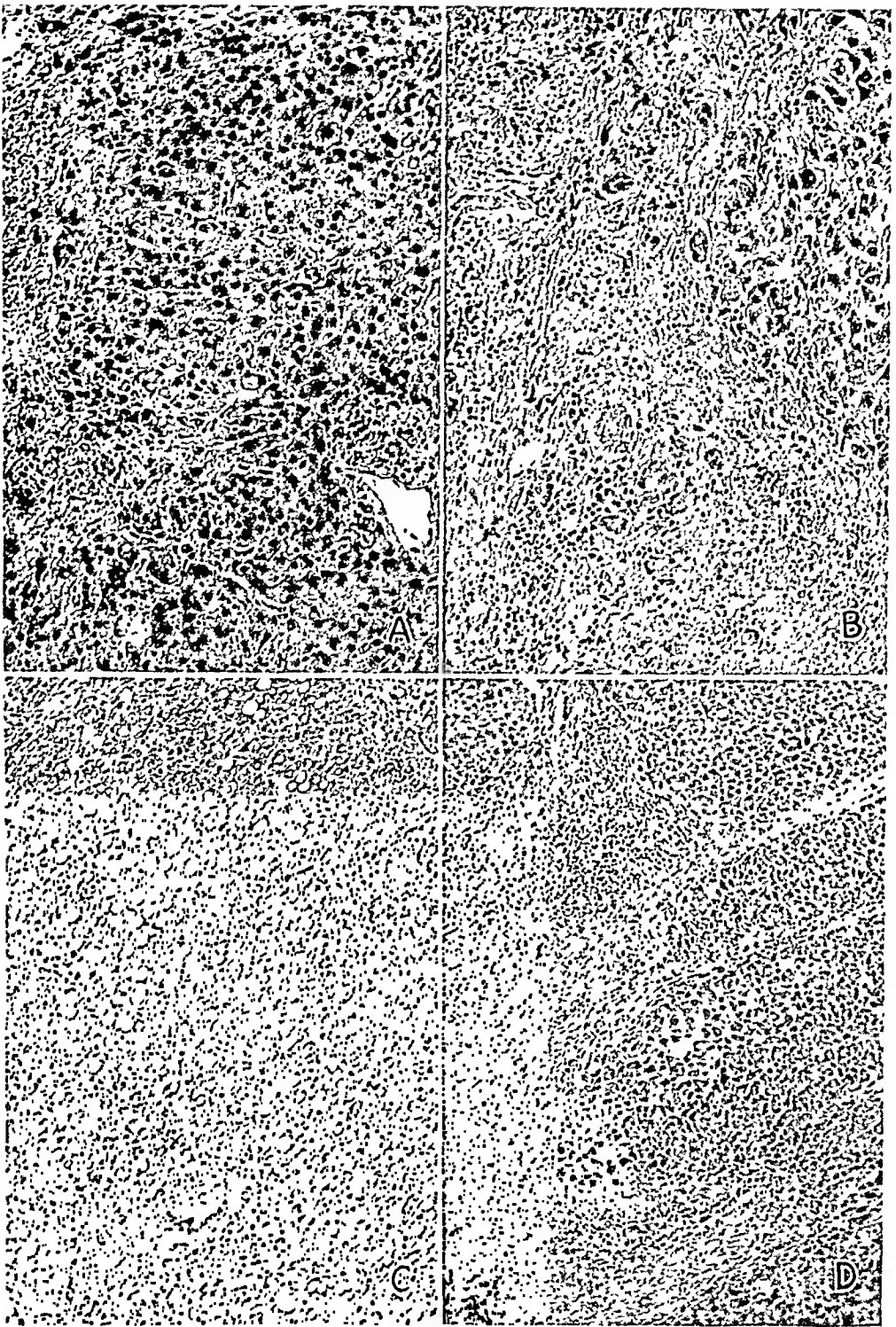


Fig. 6.—*A*, central toxic necrosis in a civilian. In the central half of the lobule, the liver cell cords are missing, and in the meshes of the collapsed connective tissue are large organized liver cell remnants and fragments without nuclear staining. There is little concentration of exudate cells in the necrotic area and the portal triads. Marked edema is noted in the intact portion of the lobule.

B, peripheral toxic necrosis in a civilian. The liver cells in the peripheral zone of the lobule have disintegrated, but large anuclear liver cell fragments are found. In the intact border zone there is dissociation of the liver cell cords.

C, fatty toxic necrosis in a civilian. Diffuse fatty metamorphosis is seen; in the central portion of the lobule the liver cells disintegrate. Here large liver cell fragments are noted without significant numbers of exudate cells.

D, chronic toxic necrosis (postnecrotic cirrhosis) in a civilian. In the wide bands separating irregularly arranged pseudolobules, moderate cellular infiltration, chiefly lymphocytes, is noted. Phagocytic elements and liver cell regenerates resembling proliferating bile ducts are absent.

the total serum protein was 6.8 Gm. per hundred cubic centimeters, and the icteric index was 64. His jaundice deepened, and he became anuric and died on the eighth hospital day, in coma. On the day prior to death the serum nonprotein nitrogen was 158 mg. per hundred cubic centimeters and the icterus index was 118.

At autopsy the liver was deeply yellow tinged, had diminished consistency and weighed 1,750 Gm. The edge was blunt and the capsule tense. The lobular pattern on cut section seemed exaggerated by marked congestion in the central areas. Histologically (fig. 6A), the liver cell cords in the central and intermediate zone were barely recognizable. Many of the liver cells had disappeared; the remaining cells failed to reveal nuclear staining, but otherwise structural details were recognized. Their cytoplasm often revealed small or large fat droplets; in addition, it contained irregularly shaped, strongly eosinophilic clumps. It was usually diffusely pigmented with bile. Also many cell fragments of similar character were noted. The Kupffer cells were proliferated and contained phagocytosed material. The sinusoids were, as a rule, wide, and occasionally fibrin thrombi were seen in them. Bile-laden inflammatory monocytes were sparsely intermixed. Only peripherally in the lobules were the liver cells fairly well preserved. The perisinusoidal spaces were widened. The portal triads revealed a few round-cellular and polymorphonuclear exudate cells.

Acute Toxic Hepatitis with Peripheral Necrosis.—A Negro woman aged 43, a factory worker, who had malaria at the age of 13, underwent, three weeks prior to being admitted to the hospital, nausea, vomiting and epigastric distress. One week later she became jaundiced, had acholic stools and dark urine. Eight months prior to this hospitalization she had three "hip shots," the nature of which was unknown. The physical examination gave essentially negative results. The icterus index was 312; the serum nonprotein nitrogen was 33 mg., the chlorides 375 mg., and the total cholesterol 208 mg. per hundred cubic centimeters; the cholesterol ester ratio was 35 per cent; the serum albumin was 3.3 Gm. and the globulin 3.0 Gm. per one hundred cubic centimeters. The urine revealed albumin (a trace), bilirubin (4 plus), no urobilinogen and an occasional white blood cell. The red blood cell count was 4,500,000 and the white cell count 13,450. Roentgenologically, a calcific density on the right side opposite the second lumbar vertebra was interpreted as "possible gallbladder or common duct stone." On the sixth hospital day, choledochostomy was performed and a rubber drain inserted. The gallbladder appeared collapsed, and a small stone was found in the fundus. The common bile duct was not dilated. The patient had hemorrhages from the mouth and from the operative wound. She gradually became worse and died on the sixteenth hospital day.

At autopsy the flabby liver weighed 770 Gm. The capsule was gray and slightly wrinkled. The cut surface was brownish red. The lobular pattern was generally obscured, and only in circumscribed areas did it appear more marked than usual. Microscopically (fig. 6B), in the central portion of the lobule, dissociation of otherwise intact liver cell cords and edema were noted. In the peripheral zone, however, the connective tissue framework was completely denuded of liver cells, producing the impression of enlarged portal triads. In the denuded areas, a few bile-laden liver cells with or without nuclear staining were noted. Many exudate cells, chiefly polymorphonuclear leukocytes, were seen in and around the lymphatic channels of the periportal fields. The bile ducts showed moderate proliferation. The areas around the central veins were normal.

Acute Fatty Hepatitis.—A 53 year old white laborer who had been epileptic for several years fell and struck his head on the ground during an epileptiform attack. The scalp wound was not serious; however, the next day he noticed a yellow

color of the skin and scleras. He became increasingly drowsy and stuporous and was admitted three days after his injury in a semicomatose condition with a temperature of 102.6 F., a pulse rate of 128, a respiratory rate of 24, moderate jaundice and rales in the lower lobe of the right lung. The liver was palpable 3 finger-breadths below the costal margin; the spleen was not palpable. The urine revealed bilirubin (4 plus), no urobilinogen, albumin (3 plus), and occasional red and white blood cells. The ieterus index was 71. The patient's stuporous condition gradually increased, and he died on the third hospital day. Despite thorough search, no evidence of intoxication of any sort was found.

At autopsy the large, dark green liver weighed 2,700 Gm.; its consistency was doughy, but soft. The edges were rounded and the capsule tense. On the greasy cut surface the markings were visible, the lobules being peripherally yellow green, with reddish centers.

Histologically (fig. 6C), small and large fat droplets replaced the cytoplasm of the liver cells diffusely. Liver cells with at least partially nonfatty cytoplasm were seen only on the lobular periphery. Throughout, but more marked in the central zone, the liver cells contained bile-stained granules and ramified perinuclear eosinophilic clumps. The sinusoids were narrow, and, especially in the central zone, scarcely any red cells were found. In the central zone the liver cells were disintegrated, and bile-stained remnants and large fragments without nuclear staining were irregularly scattered. They revealed fatty changes and clumps of coagulated protein and were surrounded by only few, if any, exudate cells, mostly of round-cellular character. The latter possibly derived from the large proliferating Kupffer cells, which contained brown-stained coagulated or fatty material in almost all parts of the lobule. There were few exudate cells in the portal triads, chiefly of polymorphonuclear character. The bile ducts revealed no changes.

Chronic Toxic Hepatitis.—A rash, jaundice and headaches developed in a 36 year old white woman after she had worked for eleven months with trinitrotoluene in a naval ordnance plant. Three other workers in the plant in whom similar symptoms developed died. In six months she had apparently recovered. She was well for four months, and then she began to complain of menorrhagia. This and gradually increasing weakness and dyspnea were her only complaints until three months later, when a generalized rash developed with epistaxis and bleeding from the gums. On admission she was markedly anemic and revealed petechiae all over the body. The mucous membranes of the mouth and gums showed evidence of bleeding. Liver, spleen and lymph nodes were palpable. The urine showed albumin (2 plus) and many red blood cells. The red blood cell count was 1,020,000, with 26.8 per cent reticulocytes; the hemoglobin content was 15 per cent; the white cell count was 4,500, with a normal differential count; the platelets were almost completely absent. After several blood transfusions, splenectomy was performed, but the patient died immediately after the operation.

At autopsy the liver weighed 1,300 Gm. and was greenish brown, with coarse, irregular granulations. Large nodules were separated by firm connective tissue bands of variable widths in which lumens of vessels were often present. In the nodules, which measured up to 2 cm. in diameter, the lobular markings were well recognized. Microscopically (fig. 6D), in the greater part of the liver the original lobular pattern appeared distorted by enlargement of the portal triads, the lymphatic channels of which were infiltrated with round and polymorphonuclear cells. In some areas, the lobular pattern was absent, and regenerated pseudolobules or nodules predominated. These likewise revealed little alteration of the liver cells themselves. Between the lobules and nodules were wide areas almost devoid of liver cells; only few necrotic cells and irregularly scattered anuclear cell fragments were found in

the collapsed connective tissue framework. There was almost no proliferation of fibers. Between them, occasionally polymorphonuclears or round exudate cells were found, which were mostly small and not phagocytic. Proliferated bile ducts were seen in the portal triads and the larger scars. They were clearly differentiated from regenerating liver cell cords by their small dark nuclei and the absence of bile casts. These areas of collapse without any sign of reconstruction were obviously the residue of preceding extensive necrotizing processes which had completely destroyed several lobules.

Comment.—The common histologic abnormalities in all 69 cases of this group are zonal necrosis and the silent denudation of the connective tissue framework. Relatively little response of mesenchymal cells, if any, is seen around the disintegrating liver cells. If present, it is at least partially and sometimes predominantly polymorphonuclear in character. The latter character is especially marked where the liver cells are still arranged in intact cords. The exudate cells reveal little phagocytosis. The Kupffer cells, however, are mobilized, and they contain phagocytosed breakdown products of liver cells. The liver cells themselves demonstrate various degrees of degeneration and disintegration. Their cytoplasm loses the normal basophilia and is eosinophilic; small and large fat droplets appear, especially in the fatty form of the disease; in addition smaller and larger clumps of strongly refractile coagulated protein are seen in the cytoplasm, usually arranged around the nucleus. They may coalesce to form larger ramified bodies resembling the hyaline material described by F. B. Mallory²⁹ in alcoholic cirrhosis. Complete coagulation necrosis of entire liver cells is seldom found. Around the coagulated clumps hydropic degeneration may be noted. In the immediate vicinity of the necrotic area the nuclei of the liver cells lose their basophilic staining or less often become pyknotic or break up. Remnants of liver cells are found which still reveal the structural organization of the liver cells but are uniformly eosinophilic or may reveal diffuse imbibition of bile pigment (ghost cells) (fig. 4 B). They break up into irregularly outlined large eosinophilic or bile-pigmented fragments in which, also, the previous structure is still suggested, indicating a slow cell death. Round, diffusely hyalinized bodies of the character of Councilman bodies were not seen in the cases examined. Regenerative processes, if any, are in the background and, when present, do not reveal a bizarre or "wild" character. Mitoses, however, are more common than they are normally, especially on the borderline of the necrotic portion. The sinusoids often contain recently formed fibrin thrombi, especially in the necrotic areas. There is marked widening of the perisinusoidal spaces, which contain albuminoid debris. Edema is outspoken in all forms but the fatty hepatitis. It is present not only in the necrotic areas, where the framework is often separated by

29. Mallory, F. B.: Bull. Johns Hopkins Hosp. 22:69, 1911.

fluid, but also in the histologically intact zone. The portal triads are hardly involved except in the peripheral or the chronic form, the cellular infiltration often including polymorphonuclear cells. The zonal arrangement is far more outspoken than in the viral form. In the fatty variety it is concealed by the diffuse fatty metamorphosis.

In the toxic group the centrolobular necrosis is the most common picture (table 4), representing a more advanced stage of the well known

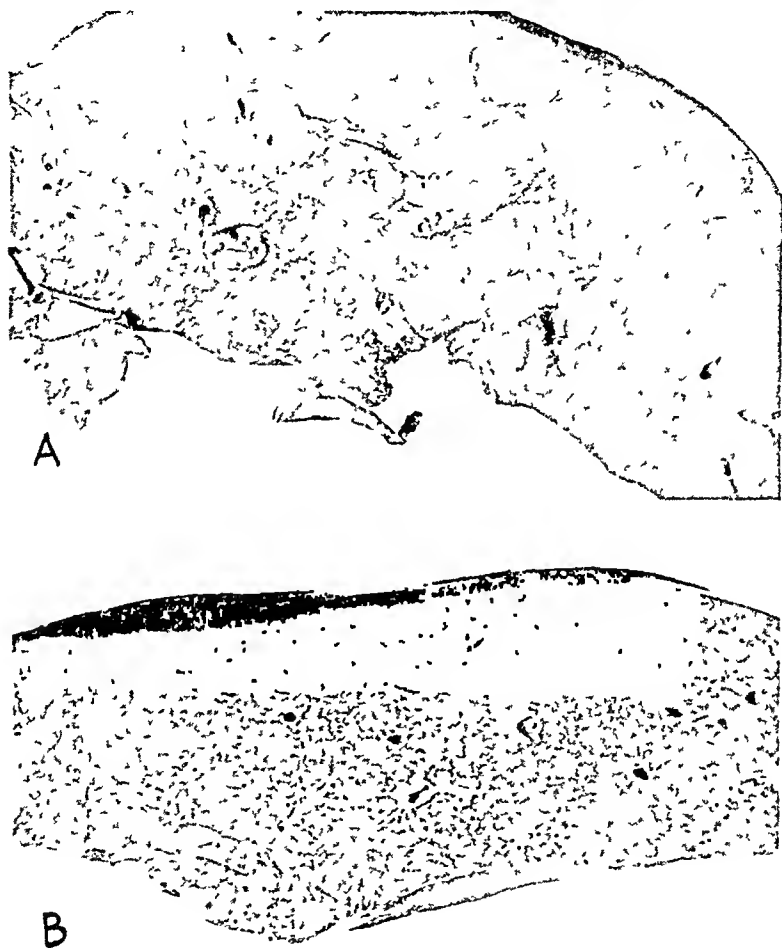


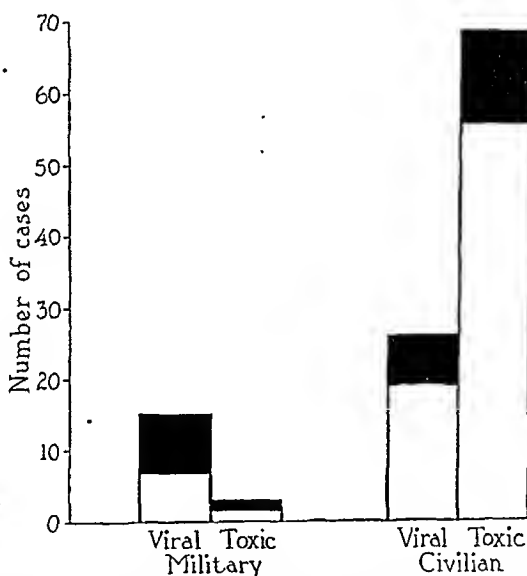
Fig. 7.—*A*, gross specimen showing acute toxic fatty necrosis. The lobular markings are obscured on the uniform-appearing cut surface.

B, gross specimen showing acute toxic central necrosis. On the cut surface the lobular markings are exaggerated

phenomenon observed at autopsy in many toxic conditions. It is followed in incidence by the fatty change, which comprises less than a fourth of the cases. In the fatty form the fatty metamorphosis is usually diffuse throughout the lobule; however, the necrotic changes which differentiate this form from simple fatty degeneration or infiltration are mostly centrolobular. Peripheral necrosis was only rarely encountered.

Necrosis of the intermediate zone was not seen in the series examined. The small number of instances of chronic hepatitis in this series is explained by the difficult and arbitrary separation from postnecrotic cirrhosis.

In the gross the liver in the acute type of fatal toxic hepatic necrosis is relatively large and usually reveals a blunt edge. The lobular markings are in many instances obscured because of the edema or because of the relatively low blood content in the fatty form (fig. 7 *A*). In other instances, especially in the presence of central necrosis (fig. 7 *B*), the increased blood content in the collapsed central areas exaggerates the lobular pattern. In small circumscribed areas the hyperemic and collapsed central fields are connected by bridges surrounding the small remnants of intact parenchyma in the periphery of the lobules. The



• Fig. 8.—Incidence of viral and toxic fatal hepatic necrosis in the studied military and civilian material. Solid black portions represent cases of known cause; white portions, cases of unknown cause.

picture then appears rather similar to subacute passive congestion of the liver. It is differentiated from it by the rather low consistency. Even in the fatty form, despite its doughy character, the consistency is diminished. The picture of the entire liver, especially on the cut surface, is rather uniform and little difference between the lobes is apparent. The disease process appears to be of equal age in the entire liver. The gross picture of the peripheral form does not vary much from that of the central one. The fatty form obviously reveals a greasy appearance.

All cases showed clinically evidence of hepatic failure and more or less severe jaundice. In only 13 of the 69 cases were etiologic factors, usually considered hepatotoxic, recorded. The average number of days between outbreak of jaundice and death was somewhat greater in the

acute central form than in the fulminant viral form; in the fatty form it was almost as short. The few cases of the peripheral form revealed a longer duration. The average age was higher in the toxic than in the viral group; sex and color distribution were not characteristic.

TABLE 5.—*Histologic Characteristics That Differentiate Between "Viral" and "Toxic" Hepatic Necrosis*

	"Viral" Type	"Toxic" Type
Distribution of damage	Diffuse, with central pre-dominance	Usually zonal
Cell death	Rapid	Gradual
Large cell remnants or fragments without nuclear staining but with shadows of cellular structure (ghost cells), often with diffuse imbibition of bile	Absent, except for rapidly disintegrating cells in very early fulminant form	Present
Cytoplasmatic clumps of coagulated protein	Usually absent	Often marked
Hyalinization of cells due to diffuse coagulation necrosis (Councilman bodies)	Present	Absent
Fatty changes	Little, if any (small droplets)	May be marked (small and large droplets)
Denudation of connective tissue framework	Reactive	Silent
Rupture of framework	Absent	Occasionally present
Edema with widening of perisinusoidal space	Absent	Outspoken except in fatty form
Cellular infiltration	Chiefly mononuclears	Less marked; polymorphonuclears may predominate
Bile-containing macrophages	Many	Few
Phagocytosis	Marked	Moderate or slight
Fibrin thrombi	Rare	Rather common
Liver cell regeneration in form of pseudo bile duct proliferation	Common	Often absent

TABLE 6.—*Gross Characteristics That Differentiate Between "Viral" and "Toxic" Hepatic Necrosis*

	"Viral" Type	"Toxic" Type
Size of liver	Usually reduced	Slightly enlarged in central necrotic form, moderately to markedly enlarged in fatty form
Uniformity of picture	Often absent	Present
Cut surface	Spleenlike or focally exaggerated lobular pattern	Diffusely obscured or exaggerated lobular pattern
Consistency	Markedly reduced	Slightly reduced
Greasy appearance	Absent	Occasionally present

COMPARISON OF THE TWO GROUPS

A comparison of the viral and the toxic group in the civilian material permits conclusions similar to those drawn in regard to the military group. The far greater number of cases studied makes the observations more valid. In the civilian material studied there is, quite unexpectedly, a definite preponderance of the histologic picture considered as toxic necrosis over that considered as viral in origin (fig. 8).

The histologic differences between the two groups have been considered previously. Table 5 represents an attempt to summarize them. The histologic differentiation in individual cases was only occasionally difficult.

Grossly, the distinction between the toxic and the viral group was not always easy; the characteristics used are summarized in table 6. In general, the liver with the viral form of hepatitis reveals a more variegated outer and cut surface than that with the toxic form, in which the variations between the different parts of the liver are far less marked and the lobular pattern in the acute form is uniform throughout. The consistency is reduced in both groups but always more in the viral hepatitis which reveals the characteristic picture of acute yellow atrophy.

TABLE 7.—*Weight of Liver and Spleen in Viral and Toxic Hepatic Necrosis*

Stage or Form	Cases in Which Weight of Liver Was Known	Weight of Liver in Gm.		Cases in Which Weight of Spleen Was Known	Weight of Spleen in Gm.	
		Average	Range		Average	Range
		Cases of "Viral" Type				
Stage:						
Acute.....	16	1,284	990-1,750	9	286	200-500
Subacute.....	11	1,076	780-1,575	8	198	90-375
Chronic.....	7	1,176	500-1,500	3	215	180-250
Totals.....	34	1,194	500-1,750	20	245	90-500
Cases of "Toxic" Type						
Form:						
Central necrosis....	24	1,560	800-3,280	16	247	90-650
Fatty necrosis.....	13	2,340	1,400-4,100	11	241	60-300
Peripheral necrosis..	2	1,450	700-2,140	2	190	175-250
Chronic.....	8	1,795	1,200-2,250	7	207	120-360
Totals.....	47	1,810	770-4,100	36	234	60-650

The liver is on the average larger in the toxic form than in the viral form. It weighs, especially in the fatty variety of the toxic form, more than normal, in contrast to the reduced weight of the liver in the viral form (table 7). This enlargement, which is indicated by the blunting of the edges, is to a great degree caused by the marked edema which characterizes the toxic form.²⁴ Fat deposition, blood engorgement and preservation of liver cells in part of the lobule are other factors responsible for the relatively large size of the liver in toxic necrosis.

The spleen may be enlarged in both groups, showing the picture of a reactive hyperplasia in the acute and subacute form and that of a fibrocongestive splenomegaly in the chronic one. No characteristic differences between viral and toxic necrosis are found.

As to changes in the abdominal lymph nodes, the available records are incomplete. In the viral group observed during the performance of this study the hyperplasia described by Lucké²⁵ was noted. This was less evident in the toxic group.

The kidneys reveal cholemic nephrosis with the characteristic bile casts in cortex and medulla and the degenerative changes of the tubules far more often in the toxic than in the viral form. Oliguria and even anuria may develop; in many instances of toxic necrosis, as in carbon tetrachloride poisoning, full-fledged low nephron nephrosis is observed.³⁰ In the fulminating viral form, fat droplets are found in the tubular cells of the cortex as described by Lucké and Mallory^{2b}; this, however, is not associated with impaired renal function or oliguria. The difference in the renal involvement between fatal toxic and viral necrosis is reflected in the generally much higher rise in the serum nonprotein nitrogen in the toxic as compared with the viral form (table 8). The differences between the viral and the toxic form in the morphologic picture of the kidney and the elevation of the nonprotein nitrogen are especially marked in the acute and less clear in the chronic stage.

TABLE 8.—*Serum Nonprotein Nitrogen in the Studied Fatal Cases of Hepatic Necrosis*

Type	Cases in Which Nonprotein Nitrogen Was Recorded	Nonprotein Nitrogen in Mg. per 100 Cc. of Serum	
		Average	Range
"Viral".....	15	42.1	17-75
"Toxic".....	25	83.7	20-232

III. COMBINATION OF VIRAL AND TOXIC CHANGES

In several instances in which the liver had the appearance of sub-acute or chronic viral necrosis, the centers of the regenerated nodules or of the preformed lobules revealed acute necrosis characteristic of the toxic type, namely, gradual disintegration of liver cells without significant mesenchymal reaction. An example of this is shown in the following case of homologous serum hepatitis:

A 71 year old white man was operated on for carcinoma of the colon, at which time he received several blood transfusions. Two months later he noticed a yellow discoloration of his skin and a dark color of his urine; his stools became clay colored. He complained of abdominal cramps, distention and anorexia. He stayed at home under a local physician's care for a week, but his condition did not improve and he was admitted to the hospital. Except for the moderate jaundice, the physical examination gave essentially negative results. The urine contained bilirubin (3 plus) and urobilinogen (1 plus). The red cell count was 3,000,000, with hemoglobin content 72 per cent; the white blood cell count and the differential count were essentially normal. His temperature rose to 101.2 F.; he gradually became stuporous and died in a coma on his seventh hospital day.

At autopsy the liver weighed 780 Gm. and was slightly softened. The irregular surface was in general yellowish red and revealed many small nodules of variable

30. Lucké, B.: *Mil. Surgeon* 99:371, 1946.

size (from 0.3 to 1 cm. in diameter), some of which were brown. The cut surface showed loss of the lobular structure. The small nodules were separated by gray trabeculae. Microscopically (fig. 9*A*), the connective tissue was collapsed in wide areas. The arrangement of the vessels, however, was preserved. Many irregularly regenerating liver cell cords, which contained bile casts in the dilated bile capillaries, were seen in the collapsed areas. The shape of these cells was usually bizarre. In the framework were also noted histiocytes and lymphocytes, both accumulating chiefly in the periportal fields. Liver cell fragments were almost absent. Irregularly scattered throughout the organ were small islets of well formed liver cells. Most of them were reconstructed pseudolobules without the cords arranged around the central vein, and only in a few places did they reveal a normal lobular structure. Both the nodules and the preformed lobules showed a circumscribed central zone

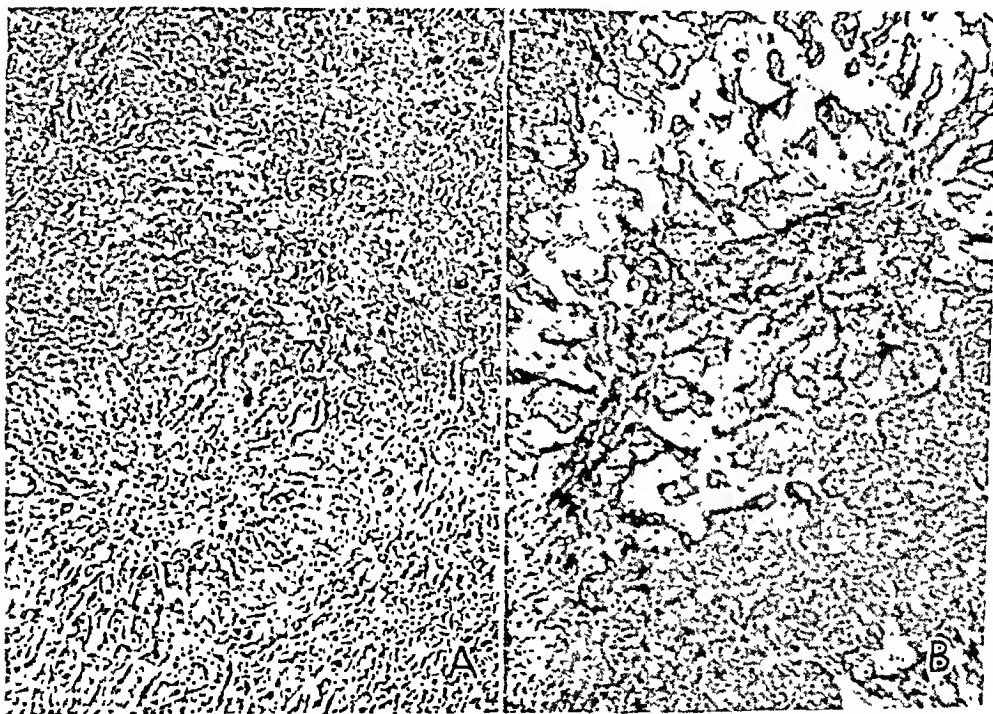


Fig. 9.—*A*, subacute viral necrosis with “toxic” necrosis in the center of a nodule. A collapsed framework containing irregular regenerating liver cell cords and mononuclear exudate cells surrounds the liver nodule. In its center there is denudation of the framework, associated with little cellular exudation but with presence of large liver cell remnants which still reveal the shadows of cellular structure.

B, central toxic necrosis with rupture of the reticulum framework (Gömöri's reticulum fiber stain). In circumscribed areas the fibers are widely separated and ruptured. This produces large blood pools.

in which the liver cells were either not present or revealed lack of nuclear staining with shadows of the cellular structure and imbibition of bile. They contained coagulated clumps of protein. Large liver cell fragments of similar character were also present. Edema and collapse of the framework were seen. However, cellular infiltration was missing, and only the Kupffer cells were proliferated. This transition between the necrobiotic and the intact areas was gradual.

Comment.—The recent central necroses occurring quite commonly in the preformed lobules or the regenerated nodules were probably the ultimate cause of death since they had destroyed part of the remaining functioning parenchyma which still had been able to maintain life. The morphologic picture speaks against a viral causation of the recent parenchymal damage but suggests the effect of toxic substances injuring an already damaged liver, and one can thus speak of secondary toxic necrosis. However, these toxic lesions are similar to those seen in passive congestion of the liver,³¹ which are obviously caused by anoxia of tissues resulting from retardation of the blood flow. Recently³² the possibility has been emphasized that central hepatic necrosis may be the result of circulatory disturbances, and especially the centrolobular necrosis in the fatty form has been explained by the obstructing of the sinusoidal blood flow by the swollen liver cells. Likewise, the centrolobular necrosis in the more chronic forms of viral necrosis may possibly be due to a retardation of the blood flowing through the sinusoids in the central part of the lobule or the nodule. This could be explained by the distortion of the vascular bed in these cirrhotic or precirrhotic conditions.³³ For blood to flow normally through all parts of all lobules of the liver it is required that the distance between tributaries of the portal veins and the central veins be identical all over the liver.³⁴ This structural principle is destroyed by irregular nodule formation. If in such a condition some minor cause interferes only slightly with the general hepatic circulation, the centers of lobules and nodules may become necrotic if the hepatic circulation was a priori disturbed by the distortion of the lobular pattern in chronic hepatitis. The central anoxic necrosis, often fatal in subacute viral hepatitis, is, therefore, not necessarily explained by persistent activity of the virus or an additional injurious substance, but is possibly caused by anoxia and can thus be a sequela of the original disease.

IV. CONNECTIVE TISSUE FRAMEWORK

The intralobular reticulum framework is intact in all forms of viral hepatitis, as clearly shown by Lucké.³⁵ Even where complete denudation and collapse of the framework occurred, the fibers revealed neither rupture nor evidence of proliferation. In the material of the toxic group, also, the framework appeared intact except for the case to be presented now.

31. Mallory, F. B.: *J. M. Research* **24**:455, 1911.

32. Himsworth, H. P.: *The Liver and Its Diseases*, Cambridge, Mass., Harvard University Press, 1947.

33. McIndoe, A. H.: *Arch. Path.* **5**:23, 1928.

34. Pfuhl, W., in Möllendorff, W.: *Handbuch der mikroskopischen Anatomie des Menschen*, Berlin, Julius Springer, 1932, vol. 6, pt. 2.

35. Lucké, B.: *Am. J. Path.* **20**:595, 1944.

A 57 year old white man was admitted to the hospital with progressive abdominal swelling of six months' duration. Two months prior to admission he had acquired a nonproductive cough and began to notice an icteric tint to his skin. At that time he also felt some abdominal discomfort, especially marked in the right upper quadrant. His past history was essentially irrelative. On examination moderate jaundice, ascites, and tenderness in the right upper quadrant of the abdomen were noted. The liver and the spleen were not palpable. The urine contained bilirubin (2 plus). The hemoglobin content was 82 per cent; the red cell count, 4,200,000 and the white cell count 11,150, with a normal differential picture; the serum non-protein nitrogen was 38 mg. and the total cholesterol 156 mg. per hundred cubic centimeters, with an ester ratio of 54.5 per cent; the alkaline phosphatase was 11.2 Bodansky units, and the icterus index was 90 units. Roentgenologic examination of the chest and the gastrointestinal tract revealed no abnormality. The patient declined rapidly, dying on the fifteenth hospital day.

At autopsy the liver was brown and weighed 1,510 Gm. It was of firm consistency and revealed a granular surface and a smooth capsule. On the cut surface the lobular markings were obscured, and an irregular fine red mottling was noted. Around some central veins a wide dark red area was found. The portal triads appeared widened. Microscopically (fig. 9B), the structure of the liver cell cords in the centers of the lobules was distorted. Individual liver cells were dissociated and were diffusely bile stained; occasionally, their cytoplasm contained refractile eosinophilic inclusions of ramified shape. In addition, some large anuclear cell fragments were present. Around them a few round or polymorphonuclear exudate cells could be seen. The bile capillaries were dilated and contained bile thrombi. The sinusoids in the central areas were in general wide; occasionally the separating connective tissue framework was ruptured. This caused large, centrally located pools of blood. In the peripheral zones the liver cells were intact but separated by edema. Peripherally in the lobules some distorted reconstruction was noted, with beginning formation of pseudolobules. The slightly fibrotic periportal and central fields revealed a few bile-laden exudate cells with few polymorphonuclear leukocytes and little bile duct proliferation.

Comment.—This case of central toxic necrosis appears to be sub-acute because of periportal and central fibrosis with nodule formation. The blood pools caused by the rupture of the reticulum framework produce a morphologic picture similar to the one described in acute allyl formate intoxication of the dog.³⁶ Whereas the older literature³⁷ generally claims that in acute atrophy of the human liver the framework is intact, one occasionally finds a report describing a rupture of the reticulum fibers.^{37a} Proliferation of the collapsed reticulum fibers in central necrosis was occasionally seen in this series; it has been described in experimental animals³⁸ and also in man.³⁹

36. Popper, H.: *Ztschr. f. klin. Med.* **131**:161, 1937.

37. (a) Maresch, R.: *Zentralbl. f. allg. Path. u. path. Anat.* **16**:641, 1905. (b) Kon, J.: *Arch. f. Entwcklungsmechn. d. Organ.* **26**:492, 1908. (c) Huzella, T.: *Verhandl. d. deutsch. path. Gesellsch* **18**:250, 1921.

38. Popper, H.: *Virchows Arch. f. path. Anat.* **298**:574, 1937.

39. Herxheimer, G.: *Beitr. z. path. Anat. u. z. allg. Path.* **43**:84, 1908; **72**:56, 1924.

TIME FACTORS

Conclusions as to the duration of the process and the speed of its progress are possible in some instances. In others, however, the correlation of the clinical and morphologic pictures is rather difficult. This is exemplified by the cases presented now.

Early Acute Toxic Hepatitis (Central Necrosis).—A 51 year old Negro attorney suffered a stroke with resulting hemiplegia four and one-half months prior to his hospitalization. Two days before admission he attempted suicide by swallowing approximately 8 ounces (273 cc.) of an antiseptic, deodorant and disinfectant mixture of coal tar phenols and oils ("creolin"). After administration of starch water he vomited a brownish liquid. At admission his temperature was 101.4 F., and he com-

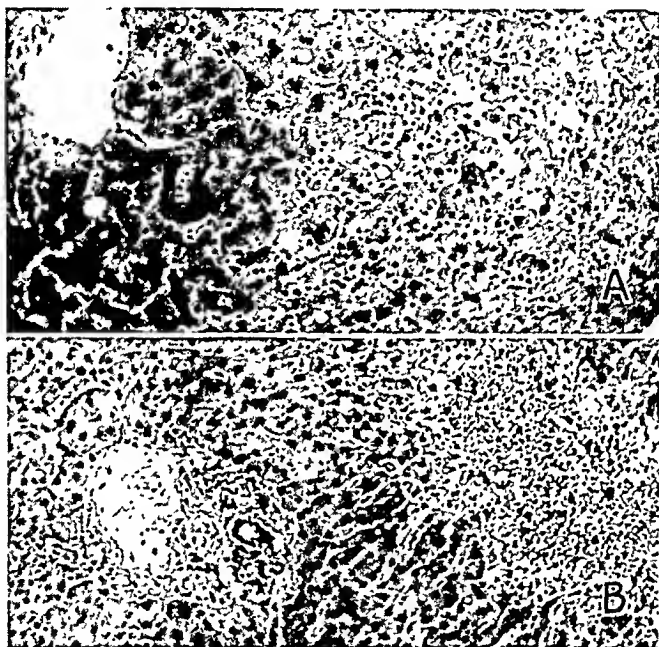


Fig. 10.—*A*, early central toxic necrosis. The arrangement of the liver cell cords of the central zone is distorted. They stain eosinophilic and reveal clumps of coagulated protein. They are surrounded by large numbers of polymorphonuclear leukocytes.

B, late central toxic necrosis. The liver cells in the central half of the lobule have mostly disappeared. In the border zone adjacent to the intact liver cells anuclear cell remnants and fragments are found. There is no fibrosis and little cellular infiltration. The portal triads reveal some polymorphonuclear leukocytes.

plained of severe dysphagia. He exhibited buccal and pharyngeal chemical burns. The scleras were icteric. The urine contained albumin (3, plus) and bilirubin (2 plus). The red cell count was 4,600,000, with hemoglobin 74 per cent; the white cell count was 11,600, with a normal differential count. The Kahn test revealed no syphilis. Despite temporary improvement on dietary and supportive management, he died six days after being admitted to the hospital.

At autopsy the firm liver weighed 1,500 Gm. The surface was smooth and brownish red. On the cut surface the lobular markings were exaggerated, producing a nutmeg appearance. Microscopically (fig. 10 *A*), in the central and intermediate

areas of the lobule the liver cells were shrunken, often dissociated and fragmented. They were eosinophilic and often revealed clumps of coagulated protein and fat droplets of various size in the cytoplasm. The nuclei were preserved. In the wide sinusoids, as well as in the dilated perisinusoidal spaces, many polymorphonuclear leukocytes were found, often surrounding necrotic liver cells. The Kupffer cells were proliferated. Toward the periphery there was noted a gradual transition to normally stained liver cells with only occasional granules of coagulated protein. The periportal fields revealed little infiltration, the cells being chiefly of polymorphonuclear character.

Comment.—In this instance, death due to hepatic insufficiency occurred eight days after the ingestion of the poison. The acute character of the necrosis of the liver cells, which was caused by a chemical substance, is indicated by the fact that the liver cells had not yet been removed and possibly also by the marked leukocytic reaction.

Late Acute Toxic Hepatitis (Central Necrosis).—A 77 year old white man was admitted with epigastric pain of three months' duration. There was progressive decrease in appetite, with loss of 6.5 Kg. (15 pounds) in weight and increasing fatigue on slight exertion. Two weeks before admission progressive jaundice developed. A tender liver was palpable 2 fingerbreadths below the right costal margin. There were moderate anemia and slight leukopenia. The urine contained albumin (1 plus), bilirubin (4 plus) and no urobilinogen. The serum nonprotein nitrogen was 71 mg., the creatinine 2.8 mg. and the total cholesterol 165 mg. per hundred cubic centimeters, with esters 45 per cent. Cephalin-cholesterol flocculation was 4 plus; thymol turbidity, 20.8 units. The stool was free of urobilinogen. Because of suggested extrahepatic biliary obstruction the patient was operated on. However, no changes of the extrahepatic bile ducts were found; the liver was small and softer than normal. His condition deteriorated after the operation. Anuria gradually developed, and the patient died in uremia three weeks after admission.

At autopsy the soft brownish green liver weighed 1,800 Gm. The capsule was smooth and shiny. The edges were sharp. On cut section the lobular structure was made out. The intrahepatic and extrahepatic bile ducts were not dilated. Histologically (fig. 10B), in the central portion of the lobules the framework was condensed and denuded, surrounding only a few isolated necrobiotic liver cells and cell fragments. There was hardly any cellular infiltration; there was only some Kupffer cell reaction. Few preserved liver cells revealed clumps of coagulated protein and fatty metamorphosis; in the often narrow peripheral zone only edema was noted. The periportal fields showed some cellular infiltration in which round and polymorphonuclear cells took part. Little, if any, fibrosis was present.

Comment.—The central toxic necrosis and the necrobiotic changes in the surrounding area appeared to be rather recent; nevertheless, the clinical history suggests a far longer duration if the initial clinical symptoms, starting three months before death, can be attributed to hepatic damage. There remains little doubt that the jaundice appearing five weeks before death was caused by the hepatic damage. The long duration of the process is possibly indicated by the fact that the lobules were often small, as shown by the short distance between central vein and portal field. A central necrosis of small diameter may thus be the

result of the disappearance of the greater part of the lobule with subsequent condensation.

In some instances of the viral variety, also, the morphologic picture suggests a duration differing from that indicated by the clinical history.

Subacute Viral Hepatitis with Fulminant Exacerbation.—A 32 year old white soldier had, while in combat overseas, an attack of jaundice associated with vomiting, anorexia and abdominal pain, which was considered infectious hepatitis. He improved quickly in the next few weeks, but when in the following months the jaundice became more marked he was sent back to the United States. On the hospital ship, four days prior to his death, he suddenly became markedly worse. His jaundice increased rapidly, and he became semicomatose and died in cholemia shortly after landing. Two days before death the icterus index was 240; blood urea nitrogen, 8.5 mg. per hundred cubic centimeters; total protein, 7.51 Gm. per hundred cubic centimeters, with a reversed albumin-globulin ratio. The urine showed bilirubin (4 plus) and no urobilinogen. There were slight anemia and leukopenia.

At autopsy the soft and flabby liver weighed 1,150 Gm. Through the smooth capsule, mottled red and yellow areas were seen. On cut section, in wide red depressed areas the lobular pattern was missing; in other yellow-colored areas it was either absent because of nodule formation or was well developed and even exaggerated. The portal triads appeared in general more closely spaced than normal. Microscopically, in part of the liver there was almost complete collapse and denudation of the connective tissue framework. In its meshes one could see mononuclear exudate cells, proliferated Kupffer cells and, chiefly in the periphery, a few proliferating liver cell cords. Liver cell fragments were absent. In other areas the normal structure of the liver cords was preserved. However, not infrequently they were arranged in nodules independent of a central vein. The cords here were distorted, elongated or compressed. Not infrequently, in the centers of the nodules or the lobules a silent denudation of the framework associated with necrobiosis of liver cells, presence of bile-laden large cell fragments and fatty changes were seen. The bile capillaries in this area were markedly dilated and filled with thrombi.

Comment.—The histologic picture is that of a subacute form of viral hepatitis with evidence of some toxic changes probably caused by marked interference with bile and blood flow. The clinical history, however, suggests a disease of low grade and tendency to recovery complicated by a preterminal rapid exacerbation. The latter may possibly be related to the ship's movement, the effect of which can be compared to the described damage resulting from exercise in infectious hepatitis.^{4e}

Comparison of the Two Groups.—At present it is difficult to estimate the duration of the disease from the morphologic picture, and further correlation by thorough analysis of individual cases is required. Even the viral form, which seems to run a more uniform course, may occasionally show a severe chronic picture morphologically in instances in which the clinical impression was that of milder disease suddenly complicated by rapid destruction of the parenchyma. The course of the toxic form appears more irregular. Almost identical histologic pictures may be produced in cases in which the duration was only a few days and in those extending over several months.

From a practical clinical point of view these observations emphasize the unpredictability of the outcome of mild forms of primary hepatic necrosis and the necessity of strict therapeutic management.

COMMENT

The selected cases presented may justify a differentiation of two forms of fatal acute primary hepatic necrosis associated with jaundice on a morphologic basis. One is characterized by rapid, almost explosive destruction of the liver cells with only slight centrolobular predominance and marked reaction of the mesenchyma. The other form reveals slow cell death, fatty changes, little mesenchymal reaction and usually marked zonal involvement.

The question of the causation and the nomenclature of these two forms is far more problematic than their morphologic differences. Cases of the first group present a picture rather similar to the one seen in cases of infectious hepatitis in the armed forces and elsewhere.³¹ The accepted hypothesis that the recent epidemics of hepatitis are viral in origin suggests a viral causation also for the civilian cases of the first form. This theory is supported by the few instances of this group in which etiologic factors such as administration of blood or plasma were demonstrated.² As to cases of the second group, exposure to substances of established hepatotoxic properties was reported in approximately one fifth of the cases, whereas in the remainder no definite etiologic factor could be established from the available, often incomplete, histories. However, the picture simulates that produced in man and animals by known hepatotoxic substances; it also represents a more advanced stage of lesions found in patients with various diseases of well recognized toxic character. Although in the majority of instances no definite causation was demonstrated, the name "toxic" may be applied to the whole group, including the cryptogenic cases. The term "toxic" is considered synonymous with "injurious" or "poisonous" and does not necessarily imply antitoxin-eliciting substances.²⁸ The possible toxic substances vary in nature and include chemical, pharmaceutical and bacterial poisons (such as those derived from pneumococci or salmonellas) and endogenous injurious substances, as those elaborated in hyperthyroidism. The number of substances reported to be hepatotoxic in man is great.^{5a} Also, anoxia is to be considered, since passive congestion³¹ or disturbances of the hepatic circulation³² produce a similar picture or at least facilitate its development.⁴⁰ That the virus of hepatitis itself may produce similar lesions can obviously not be excluded on the basis of the available material. However, the observation that a "toxic" picture commonly appears in the centers of the nodules or of the lobules in the late stages of viral hepatitis could also be explained

40. Mann, F. C., and Bollman, J. L.: *J. A. M. A.* **104**:371, 1935.

by circulatory disturbances due to the distorted reconstruction. It therefore would be anoxic and neither an additional toxic factor nor the virus would necessarily have to be considered the cause of these lesions. Further investigations, including transmission experiments, will have to decide whether or not in the later stages of the disease the activity of the necrotizing process is still due to the presence of the virus or is due to a sequela of the original process. The presented observations support the latter hypothesis.

The term "infectious" is better omitted, because certain infectious diseases, such as pneumonia or spirochetal jaundice, may cause a "toxic" appearance. F. B. Mallory spoke of infectious cirrhosis, referring to a *Bacterium coli* infection ascending through the bile ducts.⁴¹

The morphologic picture suggests that the probably epitheliotropic virus proliferates in the epithelial cells of the liver. It apparently changes their appearance and staining qualities and interferes with their function. When a certain stage of development of the pathologic process is reached, the liver cells either become hyalinized, owing to diffuse coagulation necrosis (Councilman body) or, more often, disintegrate rapidly (T. B. Mallory^{13e} speaks of autolysis) into very small fragments, which morphologically are not conspicuous. They are apparently phagocytosed and induce marked mesenchymal reaction. However, even in the subacute form of the disease the death of the individual cell is rapid and still organized larger liver cell fragments are absent. Only in very early examples of the fulminant form can those rapidly disintegrating cells be seen. One may speculate that in milder conditions a few cells disintegrate or hyalinize at one time, whereas in the fulminant, rapidly fatal form, apparently all cells "explode" simultaneously. Draining of phagocytosed material to the lymphatic channels of the portal triads possibly explains the inflammatory, chiefly perilymphatic infiltration in this area. The recovering cells regenerate in a bizarre fashion. A zonal involvement is not always conspicuous; however, the virus may be more effective and the pathologic process of different duration in various parts of the liver, which explains the variegated gross picture.

In contrast, in the toxic form the cell death seems to be slow even in the rapidly disintegrating liver. Regressive changes such as fatty metamorphosis or circumscribed coagulation of cytoplasmatic protein simultaneous with nuclear damage are conspicuous. Consequently, anuclear cell remnants or fragments revealing shadows of cellular structure (ghost cells) are seen. The larger cell fragments seem to induce less inflammation and phagocytosis than the small fragments do in the viral form. That may explain the subdued mesenchymal reaction. Only where complete cells are digested in situ do polymorphonuclear

41. MacMahon, H. E.: *Am. J. Path.* 7:77, 1931. Mallory.²⁹

leukocytes move in, which are in the background in the viral form. Circulatory disturbances, especially in the center of the lobule, seem to be an important part of the picture, and fibrinous thrombi are often seen. The characteristic zonal distribution of the toxic form may in some cases be explained by reduced oxygen tension of the central areas which interferes with destruction and neutralization of some injurious substances. In other instances, which are much less common in this series, the higher concentration of the injurious substances in the peripheral part of the lobule leads to peripheral necrosis without influence of the oxygen tension.⁴² The anatomic picture is, in general, well in keeping with an effect of chemical injurious substances interfering with well defined phases of hepatocellular metabolism or with disturbances of circulation, possibly caused by them.³² As a rule, in this form, a uniform gross picture is seen, since the different lobules are, equally and simultaneously involved. It should be emphasized that many changes seen in the toxic form are identical with those seen as results of postmortal autolysis. Further studies are required to determine what is contributed to these changes by premortal, agonal or even postmortal processes.

The cytologic differences between the two groups, especially as to the nature of the death of the cells and their pigmentation, deserve to be clarified further by investigations that can be made only with elaborate histochemical and histophysical methods. On the basis of the material studied so far, in contrast to the Councilman body-like cell, in the ghost cell, characteristic of the toxic form, clumps of coagulation necrosis, if present and even if coalesced to form ramified bodies, are arranged around the nucleus, and the shadow of the structure of the cell is made out. The differences seem to be the same as those between Councilman bodies of yellow fever and hyaline bodies of alcoholic cirrhosis, as pointed out by Ash and Spitz⁴³; nevertheless, further investigations would be desirable.

A more intensive study comparing the chronic stages of toxic and viral necrosis appears indicated; however, this could be better carried out in the framework of a study of cirrhosis.

On the presented basis, both lesions, viral and toxic, represent primarily epithelial involvement, with the mesenchymal reaction being apparently an expression of the speed and the character of the epithelial necrosis. From this point of view the term "hepatitis" does not really apply to either of the groups except in the sense of a parenchymatous type of inflammation. It may be still more appropriate to the viral

42. MacCallum, W. G.: *A Text-Book of Pathology*, ed. 7, Philadelphia, W. B. Saunders Company, 1940.

43. Ash, J. E., and Spitz, S.: *Pathology of Tropical Diseases*, Philadelphia, W. B. Saunders Company, 1945.

form because of the conspicuous mesenchymal reaction. In most instances of the toxic form, however, the mesenchymal reaction is so limited that the term "fatty degeneration" or "central necrosis" has been usually applied as a morphologic diagnosis.

The nomenclature of the entire group is therefore problematic. The name "hepatosis" has been recommended for conditions with primary epithelial involvement⁴⁴ but has not become popular. Non-committal terms, such as "hepatic necrosis" or "hepatopathy" would be the most correct from the anatomic point of view. Since, however, clinically the term "hepatitis" is applied to these conditions which reveal rather similar clinical and laboratory manifestations, it may in some instances be practical to speak of viral hepatitis and toxic hepatitis instead of hepatic necrosis.

Obviously, only with great hesitation can a morphologic classification of hepatic necrosis be suggested as long as the etiologic factors cannot be demonstrated in individual cases. This classification should, therefore, be considered as tentative and as a working hypothesis which may not cover all cases until more reliable methods of demonstrating viruses and chemical agents are available. The great majority of cases may fall into this classification. Nevertheless, one has to expect some instances which will not follow the presented rule. A few cases were observed which revealed features of both the toxic and the viral group; however, the characteristics of one group predominated. For instance, in a liver showing in general the viral characteristics there may occasionally be found fatty changes and some spotty coagulation necrosis. This can easily be explained by the effect of complicating factors independent of the virus. In addition, the chief difference between the two groups is, in the final analysis, the speed of the necrosis of the liver cells and transitions may occur. Thus it may be possible that in some individual patient a toxin may cause as rapid disintegration of cells as is usually caused by the virus. For instance, cases of toxic hepatitis due to sulfonamides are reported⁴⁵ which, according to the description given, would fall into the viral group. In our material the hepatic necrosis following sulfonamide therapy revealed the "toxic" picture.

All our cases in which an etiologic factor was elicited followed the rules outlined. Even established etiologic factors, however, are not easily interpreted; for instance, antisyphilitic administration of arsenical compounds is known to produce hepatic necrosis.⁴⁶ It is

44. Roessle, R.: Entzündungen der Leber, in Henke, F., and Lubarsch, O.: *Handbuch der speziellen pathologischen Anatomie und Histologie*, Berlin, Julius Springer, 1930, vol. 5, pt. 1.

45. Berger, S. S., and Applebaum, H. S.: *J. Lab. & Clin. Med.* **26**:785, 1941.
Herbut, P. A., and Scaricciottoli, T. M.: *Arch. Path.* **40**:94, 1945.

46. Wile, V. J., and Sams, W. M.: *Am. J. M. Sc.* **187**:297, 1934.

now accepted that such treatment may lead to either a toxic form, caused by arsenic, or to the viral form, homologous serum hepatitis, produced when infected blood is transmitted by the syringe during injection of the arsenical preparation. The first form occurs a few days after the injection, whereas the latter may start after several months. Of our 4 patients with a history of arsenical therapy, 2 showed the pattern of viral and 2 that of toxic hepatic necrosis. This explains why the picture of hepatitis following antisyphilitic treatment may be identical with that due to infectious (viral) hepatitis.⁴⁷

The presented interpretations, not the findings, are at variance with the recent, fascinating treatises of Himsworth.³² What has been called "viral" necrosis in this study corresponds with his massive necrosis. He considers it the result of a delayed effect, chiefly due to malnutrition. What we have called "toxic" necrosis corresponds with his zonal necrosis. Himsworth deserves much credit for pointing out the important role of (sometimes conditioned) malnutrition and of disturbances of circulation. He emphasizes more the extent of the injury and reactions inherent in the liver itself than the etiologic factors in differentiating the two groups. As already stressed, the two forms presented here are not necessarily different etiologic entities. A classification based on morphologic criteria can only suggest causes and cannot be final; it is at best a challenge and stimulus for further discussion.

The incidence of toxic and viral hepatic necrosis may be distorted in the civilian material studied. This material revealed, in contrast with the military material, a striking preponderance of the toxic group. However, before concluding that in the majority of civilian cases fatal primary hepatic necrosis or hepatitis are toxic and not viral in origin, one should keep in mind that in the patients of a large city hospital alcoholism, malnutrition and exposure to hepatotoxic substances may be more prevalent than in the population in general. Moreover, studies based on biopsies performed on the livers of a small number of patients in the same hospital revealed an approximately equal incidence of both forms in cases of benign hepatitis.⁴⁸ It appears, therefore, that the toxic form has a relatively higher mortality rate, which may explain the high incidence found at the autopsy table.

The correlation of the clinical and laboratory findings, on one hand, and the morphologic picture, on the other, is still rather hazy. In all cases there was evidence of hepatic failure and jaundice; however, the duration and the severity of the process were not always apparent from the morphologic picture, especially in cases of the toxic form. In some instances in the toxic group death occurred with destruction

47. Dible, J. H., and McMichael, J.: *Brit. J. Ven. Dis.* **19**:102, 1943.

48. Popper, H., and Franklin, M.: *J.A.M.A.* **137**:230, 1948.

of a relatively small part of the lobule, whereas in others the entire lobule had disintegrated. The concomitant involvement of other organs, especially of the kidney, with subsequent uremia, may offer a possible explanation for this discrepancy. Further studies of clinico-pathologic correlation appear indicated.

A differentiation of the morphologic aspects of the toxic and the viral form of fatal hepatic necrosis is significant, for it may lead to differentiation of two clinical entities. It might be possible to use the morphologic criteria derived from the study of autopsy specimens in the interpretation of biopsy specimens, though with some caution in view of obvious differences between postmortem and biopsy material. Autopsy and biopsy results might be used to differentiate between viral and toxic hepatic necrosis clinically and in the laboratory. Subsequently, the differentiation of toxic and viral hepatic necrosis or hepatitis may emerge from the status of an academic problem into that of prognostic and therapeutic implications.

SUMMARY

The livers of patients dying of acute primary hepatic necrosis with jaundice in the past nineteen years in a large civilian institution (histologic material was available in 95 out of a total of 136 cases) were compared grossly and microscopically with those of a much smaller number of Army personnel. Among 18 of the military specimens 15 revealed various stages of epidemic hepatitis (as described by Lucké^{2a}), whereas 3 revealed a different histologic picture. Among 95 civilian specimens 26 showed changes comparable to those in the majority of the livers from Army personnel, whereas 69 revealed central, peripheral or fatty necrosis. The histories elicited from the patients of the latter group indicated that one fifth had been exposed to substances known to be hepatotoxic. The hepatitis of the former group was considered viral in origin, whereas that of the latter was called toxic because it was morphologically similar to the pathologic process observed in man and animals following exposure to substances established as hepatotoxic. Tentative acceptance of two etiologically different groups is suggested, and characteristic morphologic differences are demonstrated, which may be briefly presented as follows:

The "viral" form, which spreads irregularly throughout the liver, reveals sudden death of the liver cells. The cells break up into small fragments or hyalinize diffusely: this process is associated with an energetic mesenchymal reaction involving chiefly mononuclear phagocytosing cells. In the "toxic" form, in contrast, zonal arrangement is more prominent, the death of cells is gradual and large anuclear cell remnants or fragments are visible, which show shadows of cellular structure; the mesenchymal reaction is subdued, and the inflammatory

cells, if present, are often polymorphonuclear; fatty changes are common. The liver is usually larger in the toxic than in the viral form. Renal damage, with azotemia, is more marked in the acute toxic than in the acute viral form.

In chronic phases of the viral form, "toxic" manifestations may appear in the preserved parenchyma, which may be due to anoxia caused by disturbance of the blood flow.

The clinical manifestations of the two forms are briefly referred to, and the difficulty encountered in clinical-pathologic correlation, especially in the "toxic" group, is emphasized. An attempt is made to explain the difference of the morphologic pictures by different mechanisms and speeds of the "viral" and the "toxic" injurious process. Future studies should reveal the incidence of the "toxic" variety in nonfatal cases, since the latter seems to have a higher mortality rate. Although the term "hepatitis" as used for these forms of hepatic necrosis appears unsatisfactory from a pathologic standpoint, it may for the time being be retained because of its widespread clinical use.

HEPATIC HETEROTOPY IN THE SPLENIC CAPSULE

GEORGE J. HEID Jr. M.D.

AND

EMMERICH VON HAAM, M.D.

COLUMBUS, OHIO

SUBSEQUENT to finding an accessory liver attached to a human gallbladder, Cullen¹ extensively reviewed hepatic anomalies reported, as far back as 1767. In his opinion anomalies of the liver are infrequent, and, among them isolated nodules of hepatic tissue are "exceedingly rare." Such isolated nodules have been found in the suspensory ligaments of the liver,¹ in Glisson's capsule,² in the wall of the gallbladder³ and scattered over the peritoneum.² Insofar as we have been able to determine from the literature⁴ compiled subsequent to Cullen's review, there is no report of hepatic heterotopy associated with the spleen.

The following case is not presented as one proved to be an instance of isolated hepatic tissue. Like Gardener,¹ who accidentally discovered hepatic tissue in an adrenal gland, we observed a focus of hepatic tissue in the capsule of a human spleen while examining slides prepared from material obtained at an autopsy.

REPORT OF A CASE

A Negro laborer, aged 59, died suddenly while at work. Death was caused by massive subdural hemorrhage associated with severe cerebral arteriosclerosis. No external or internal congenital abnormalities were noted. The left ventricle of the heart was concentrically hypertrophic. Extensive dense fibrous adhesions were present in both pleural cavities. No adhesions were seen in the abdominal cavity. The viscera, including the liver and the spleen, had been perforated by the embalmer's trochar. The liver weighed 1,850 Gm. Its external and sectioned surfaces showed no pathologic changes.

The spleen weighed 225 Gm. Located centrally on the diaphragmatic surface of the capsule was a slightly roughened irregular gray opaque area, 5 by 4 cm. On sectioning, this area resembled fibrous thickening of the capsule and was sharply demarcated from the subjacent pulp. A few small openings, resem-

From the Department of Pathology, Ohio State University.

1. Cullen, T. S.: *Arch. Surg.* **11**:718, 1925.

2. Cited by Ewing, J.: *Neoplastic Diseases: A Treatise on Tumors*, Philadelphia, W. B. Saunders Company, 1942, p. 741.

3. Thorsness, E. T.: *Am. J. Clin. Path.* **11**:878, 1941.

4. *Quarterly Cumulative Index Medicus*, 1925 to June 1946, inclusive.

bling lumens of blood vessels, were seen in the sectioned surface of the thickened area. A small block of tissue was saved for microscopic study.

Microscopic Study of the Spleen.—The peritoneal surface of the thickened area of the capsule was covered by a single layer of mesothelial cells. The mesothelial cells rested in a narrow zone of collagenous connective tissue arranged in coarse bundles. Overlying the splenic pulp was a broader zone of collagenous connective tissue. Between the inner (juxtasplic) and the outer (juxtamesothelial) zone of connective tissue were groups of well differentiated liver cells, prominent bile ducts, blood vessels and small nerves. These structures were supported by a rather loosely arranged collagenous stroma, which was replacing the liver cells in some regions.

Most of the liver cells were located nearer the juxtamesothelial zone of connective tissue. Although the liver cells were arranged in cords, separated by sinusoids, they did not form the typical lobular pattern. Some sinusoids contained erythrocytes. No bile canaliculi were observed.

Small arteries and veins, each surrounded by a broad collar of connective tissue, were located within and at the borders of the groups of liver cells. No definite correlation existed between these vessels and the hepatic sinusoids.

Most of the bile ducts were conspicuously located between the liver cells and the juxtasplic zone of connective tissue. The prominent ducts were lined by a single layer of tall columnar cells with basally located nuclei. In one large duct the epithelium formed papillary folds. A conspicuous part of each prominent bile duct was a broad collar of collagenous connective tissue on which the epithelium rested. Intramurally located in some of the "collars" were minute secondary ducts lined by small cuboidal cells. In specially stained sections, elastic tissue was inconspicuous, and muscle fibers were absent from the bile ducts. There was no obvious connection or anatomic lobular relationship between the bile ducts and the liver cells.

The few small nerves present in the supporting stroma resembled sympathetic fibers.

COMMENT

Whether this heterotopic hepatic tissue was attached to the spleen prenatally or postnatally is problematic. However, our sections contained some of the histologic features described by Cameron and Oakley⁵ as observed in autoplasmic liver transplants in albino rats. After thirty days their transplants contained regions of regenerating liver cells and bile ducts. The liver cells formed prominent sinusoids. Distinctly separated from the liver cells (proved by reconstruction) were bile ducts "ringed around" by concentric layers of collagenous connective tissue. "Budding" of the larger bile ducts was noted. Both bile ducts and liver cells were supported by fibroblasts and coarse collagen.

Regenerating liver cells were not present in our sections of heterotopic liver. Distinct separation of liver cells and bile ducts was observed. The arrangement of the liver cell groups with their sinusoids was similar. "Ringed" and budding bile ducts were present.

5. Cameron, G. R., and Oakley, C. L.: J. Path. & Bact. 38:17, 1934.

Cameron and Oakley⁵ did not observe sympathetic nerve fibers in their sections of autoplasmic transplants. In our case the presence of nerve fibers is evidence against a neoplastic origin of the heterotopic liver found in the splenic capsule, for it is generally agreed that the finding of well differentiated sympathetic nerve fibers in a nodule of tissue is evidence against its neoplastic origin (Thorsness⁸).

SUMMARY

A case of hepatic heterotopy involving the splenic capsule is described. Because nerve fibers occurred in the heterotopic liver tissue, it must be assumed that the heterotopic tissue did not constitute a neoplasm and was of antenatal origin.

MEDIONECROSIS OF THE AORTA

GEORGE D. AMROMIN, M.D.

JAKUB G. SCHLICHTER, M.D.

AND

A. J. L. SOLWAY, M.D.

CHICAGO

VARIOUS etiologic moments have been held responsible for idiopathic medionecrosis of the aorta since it was first described by Gsell¹ and Erdheim.² The work of Erdheim² is fundamental and still outstanding. He reviewed the possible etiologic factors and showed preference for some form of hyperadrenalism. He described in great detail the morphologic aspects of the lesion and noted specifically that in those instances in which the typical picture of medial necrosis was shown, vasa vasorum were almost completely absent at the site of rupture. Elsewhere, in the comparatively normal aorta below, these vessels were relatively sparse. In other instances, or where vessels could be found, they showed some degree of hyalinization. Despite this and the later work of Weise,³ he expressed the belief that the vasa vasorum did not play a role in the production of medionecrosis of the aorta. Later workers⁴ also noted alterations of the vasa vasorum.

In routine histologic studies of dissecting aneurysms of the aorta due to so-called idiopathic medionecrosis, we became aware of changes occurring in the vasa vasorum. Thus it was thought of interest to review 12 instances of medionecrosis of the aorta with particular reference to alterations of the vasa vasorum.

MATERIALS

The clinical findings were not contributory. It may be mentioned that the group consisted of 9 men and 3 women, whose ages varied from 26 to 70 years, with an average of 50 years. A history of long-standing hypertension was obtained

From the Departments of Pathology and Cardiovascular Research, Michael Reese Hospital. These departments are in part supported by the Michael Reese Research Foundation.

This study was aided by grants from the Ira Frank Fund and the A. D. Nast Fund for Cardiovascular Research. It was in part supported by the Life Insurance Medical Research Fund.

1. Gsell, O.: *Virchows Arch. f. path. Anat.* **270**:1, 1928.

2. Erdheim, J.: (a) *Virchows Arch. f. path. Anat.* **273**:454, 1929; (b) **276**:187, 1930.

3. Weise, W.: *Beitr. z. path. Anat. u. z. allg. Path.* **93**:238, 1934.

4. Moritz, A. R.: *Am. J. Path.* **8**:717, 1932. Rottino, A.: *Arch. Path.* **27**:320, 1939; **28**:1 and 377, 1939. Rottino, A., and Poppiti, R.: *ibid.* **36**:201, 1943.

from only 1 patient. Some degree of elevation of the diastolic blood pressure with or without systolic elevation was present in 6 others.

Associated postmortem findings were limited to the heart, the aorta and the kidneys.

Cardiac hypertrophy was present in almost every instance. In some it was minimal; in others, pronounced. The cardiac weights varied from 350 to 1,000 Gm. and averaged 522 Gm. The hypertrophy involved principally the left ventricle.

Coronary arteriosclerosis was present in most of these cases. It varied from slight atheromatosis to marked sclerosis and calcifications, with old occlusions. Hemopericardium, due to rupture of the aneurysm, with blood escaping into the pericardial sac, was present in 6.

Valvular changes were found in 3 cases; in 2 these were interpreted as healed endocarditis of the mitral and aortic valves. In the remaining case the mitral and the aortic valve were extremely thick and sclerotic, the latter having only two cusps and being covered with calcific excrescences. In those in which the valvular alterations were interpreted as healed endocarditis there were superimposed bacterial vegetations, acute in one and subacute in the other.

Medionecrosis of the aorta answering to Erdheim's and Gsell's criteria was present in all aortas examined. Two of these in addition had moderate to marked atheromatosis and arteriosclerosis; another, only extensive atheromatosis.

Two of the dissecting aneurysms occurred in aortas with isthmus stenosis. One of the patients was 26 years and the other 29 years old. The heart of the former weighed 530 Gm. and that of the latter 400 Gm.

The kidneys of 3 patients were normal. In those of the remainder, however, changes were present. Arteriolonephrosclerosis was present in the kidneys of 7, 2 of whom also had evidence of chronic pyelonephritis. Interestingly enough, previous unilateral nephrectomies had been performed in 2 instances. Only arteriosclerotic scars were noted in the kidneys of 2 patients. A similar number of patients had hemorrhagic infarctions of the kidneys secondary to lodgment of emboli arising from endocardial vegetations.

METHODS

Whenever possible, numerous blocks of aortic tissue were examined. These were taken not only from the sites of rupture or perforation but from relatively uninvolved zones of the aortas as well. Sections were routinely stained with Delafield's hematoxylin and eosin, and, whenever feasible, with orcein and Masson's trichrome stain.

In an attempt to demonstrate abnormalities in the vascularization of the aorta it was thought that the simplest method would be to inject the vasa vasorum with a radio-opaque material and then subject the aorta to roentgen examination. This procedure had previously been tried with aortas removed from dogs^{5a} as well as with normal human aortas. By this means, variations of the vasa vasorum of the aorta have been demonstrated between animals of different species. Additional variations have been noted^{5b} among individuals of the same species.

We were able to inject the vasa vasorum in 1 instance of dissecting aneurysm. The injection was performed at the autopsy table before the heart and the aorta were opened. The specimen was then opened by the usual technic, the aorta detached and a roentgenogram made. The distribution of the vasa vasorum is shown in figure 5, compared with that in a normal human control (fig. 4). It is

5. Schlichter, J. G.: (a) *Am. Heart J.* 32:770, 1946; (b) 35:850, 1948.

apparent that there is no filling of vessels over one particular area at the base. This zone corresponded to the grossly discernible site of tear in the intima and was near the zone of perforation where blood escaped into the pericardial cavity. Microscopically, almost all the vasa vasorum were seen to be filled with the dye. Those which were not were near the site of perforation and had markedly narrowed slitlike or stellate lumens. Narrowing was produced by medial hyperplasia and intimal proliferation. We believe that this method of demonstrating abnormalities in the vasa should be used in every instance of dissecting aneurysm and isthmic stenosis of the aorta.

OBSERVATIONS

Gross Findings.—Dissecting aneurysms were present in every aorta. However, in only 2 aortas could medionecrosis be suspected on gross examination. These revealed pale blue intimal discolorations and puckerings. Their media appeared thin and more translucent than normal.

Microscopic Observations.—Alterations of the aortic media with cystic zones, fulfilling all the established criteria for idiopathic cystic necrosis, were found in all 12 aortas. However, of most interest were the changes in the vasa vasorum.

Alterations were encountered in the vasa vasorum of 7 of the 12 aortas. They varied in type and severity. Changes were present in small and larger vessels of the adventitia. Whatever the morphologic picture, marked narrowing of the lumen seemed to be the end result. Some smaller arteries, as well as arterioles, showed principally marked hypertrophy of the media (figs. 1 and 2). Others, in addition, were narrowed by profound splitting and reduplication of the internal elastic membranes with or without subintimal deposition of lipids and hyaline substance (figs. 1 and 3). In several, the splitting of the internal elastic membrane was accompanied by clumping and distortion of the fibers, so that a continuous membrane no longer existed. Lumens were frequently reduced to narrow transverse or stellate slits. Narrowing of the smallest arterioles was frequently accomplished through hyperplasia or swelling of endothelial cells, in addition to medial hypertrophy. In 2 aortas vasa vasorum were rarely seen. When they could be found, they showed profound alterations in their media and intima. In another aorta, in addition to medial hypertrophy and intimal hyperplasia, several smaller arteries in the adventitia were involved by a collagenous type of degeneration involving all coats but not accompanied by an inflammatory reaction.

Inflammatory cells were found in the adventitia of 7 of the 12 aortas. The exudate varied from small accumulations of lymphocytes and plasma cells, some perivascular, to extensive and rather dense aggregates of lymphocytes, polymorphonuclear leukocytes, macrophages and fibroblasts. Several apparently were the results of slow dissection with the formation of granulation tissue (fig. 1).

In regard to 2 aortas with narrowings of the vasa vasorum, the possibility of rheumatic arteritis was considered, in one instance because of typical associated endocardial changes, and in the other because of the abundance of large histiocytes, some multinucleated, about the vasa vasorum.

Since many patients with dissecting aneurysm have vascular hypertension, a recent study of the aortas of 40 patients with hypertension⁶ is of interest. Twenty-three of the 40 aortas showed changes in the adventitia with frequent accumulations of lymphocytes. In 35 per cent, however, there was hypertrophy of the media of the vasa vasorum and occasional luminal narrowings due to subendothelial depositions of hyaline substance. For the most part these findings are in accord with ours.

6. Ashworth, C. T., and Haynes, D. M.: *Am. J. Path.* 24:195, 1948.

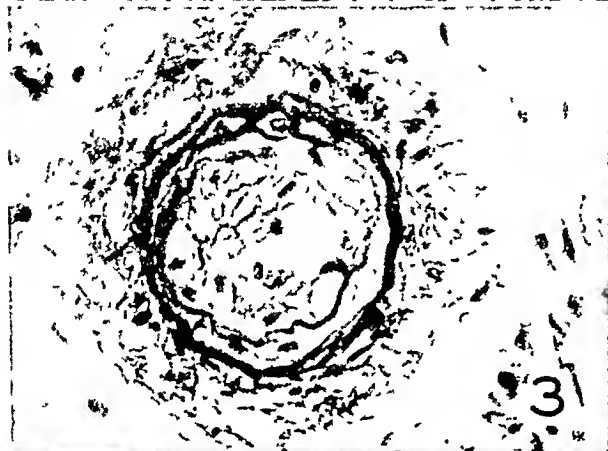
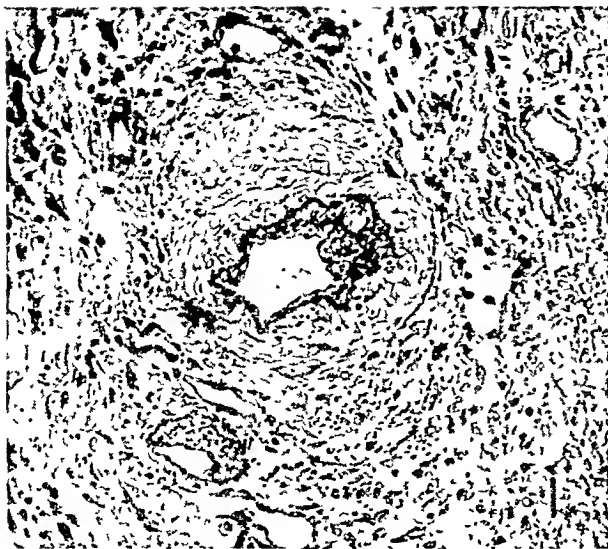


Fig. 1.—Hypertrophy of the media and reduplication of the internal elastic membrane of a small artery surrounded by granulation tissue. Orcein and iron-hematoxylin stain; $\times 130$.

Fig. 2.—Marked hypertrophy of the media and reduplication of the internal elastic membrane, with almost complete obliteration of the lumen, of a small artery. Orcein and iron-hematoxylin stain; $\times 130$.

Fig. 3.—Profound luminal narrowing caused by endothelial proliferation and reduplication of the internal elastic membrane of a small artery. Orcein and iron-hematoxylin stain; $\times 279$.

Fig. 4.—Roentgenogram of the vasa vasorum at the root of a normal human aorta, which were injected with radiopaque material. The aortic cusps are at the bottom of the illustration.

Fig. 5.—Roentgenogram of the vasa vasorum of the root and arch of the aorta in a patient with a dissecting aneurysm. Note the lack of filling of the vasa vasorum a short distance above the cusps. This zone corresponded to the site of rupture of the dissecting aneurysm and the most severe histologic alterations of the vasa vasorum.

For purposes of comparison we examined the ascending aortas from 20 patients who were normotensive and whose ages averaged 53 years. Hyalinization of the media of the vasa vasorum without luminal narrowings was encountered in 5 patients aged 61, 61, 66, 72 and 76 years, respectively. In the aortas of 3 other patients, aged 50, 66 and 73 years, respectively, narrowings of the lumens of the vasa vasorum in addition to medial or intimal hyperplasia were found. Luminal narrowings of a group of small arteries of the vasa vasorum of the 73 year old patient were at the immediate site of a large arteriosclerotic plaque which impinged on and disrupted the inner third of the media of the aorta. With exclusion of this patient, only 2 of the 20 routine controls had narrowings of the vasa vasorum without medial or pronounced intimal changes in the aorta. These alterations were less pronounced and extensive as compared with those of the group with dissecting aneurysms.

COMMENT

We encountered pathologic changes in the vasa vasorum of 7 of our patients with dissecting aneurysm. They varied from slight to pronounced arteriolosclerosis and arteriosclerosis with hypertrophy or hyperplasia of media and intima. In all, luminal narrowings were present. Abnormalities of the distribution and of the filling of the vascular bed were demonstrated in 1 aorta at autopsy by injecting the vasa vasorum with a radiopaque material. It is conceivable that the alterations of the vasa vasorum alone may result in anoxia of the aortic media.

Hypertension of vessels seems important in several respects. It may be the cause of hyperplastic intimal changes of small arteries in some instances. These changes may be the immediate or an important contributory cause of medionecrosis. After the medionecrosis has been established, even in persons whose blood vessels are normotensive, a transitory hypertension such as that produced by physical or emotional stress may increase the spread of the dissection as well as increase the chance of rupture.

As a result of ischemia of portions of the aorta, the media undergoes infarctions, or necrosis. This may manifest itself as zones of loss of structure and gradual accumulations of loose connective tissue. Some of these lesions may heal as described by Erdheim; others may become the seat of continued dissection, with hemorrhage due to tearing of blood vessels of the aortic media. "Zones of ischemia" of the aorta are suggested by those authors who believe that dissecting aneurysms may result from rupture of the vasa vasorum. Such a mechanism, i. e., vasospasm with necrosis of the distal portion of the vessel, may play a role in some patients with hypertension but without histologic alterations in the vasa vasorum.

Medionecrosis of the aorta or of peripheral vessels has been produced experimentally by chemical or mechanical means that interfere with the vascularization as carried out through the vasa vasorum of the adven-

titia in rabbits and, more recently, in dogs.⁷ The importance of the nutrition of the aorta which is carried on by way of the vasa vasorum was demonstrated by one of us, by means of coagulation of the adventitia of the ascending aorta, in dogs.⁷

As one reviews our cases and the voluminous literature on dissecting aneurysm of the aorta, it becomes apparent that this disease is not an entity in itself but is the result of varying physiologic and morphologic alterations of the wall of the aorta. The pathologic picture varies with the etiologic factor. All cases, however, have one feature in common, i. e., the destruction of the aortic media. The aorta so damaged may yield to the normal or to the increased intra-aortic pressure with spontaneous rupture or with the formation of a dissecting aneurysm and rupture.

In view of the accumulated experimental and morphologic evidence it may be suggested that the factor responsible for idiopathic cystic necrosis of the aorta is ischemia of the media. This can be produced by pathologic changes of the vasa vasorum resulting in narrowing of their lumens. Such alterations may be due to a localized variety of arteriolosclerosis or arteriosclerosis and may or may not be associated with similar changes occurring elsewhere, particularly in the kidneys.

SUMMARY

Twelve dissecting aneurysms of the aorta associated with "idiopathic" medionecrosis were studied. Narrowings of the lumens were found in the vasa vasorum of 7. Ischemia of the media of the aorta was implicated as the underlying primary factor in the production of medionecrosis.

7. Schlichter, J. G.: Arch. Path. 42:182, 1946.

MAMMARY LIPOMA

C. G. TEDESCHI, M.D.

FRAMINGHAM, MASS.

IN AN extensive series of cases of tumor of the breast, critically analyzed, Haagensen¹ found that a number of conditions considered clinically to be cancerous were shown by histologic examination to be various changes of fat tissue. In one of these cases the breast had been unnecessarily removed. Commenting on this unfortunate occurrence, Haagensen recalled a similar case reported by Keynes,² in which a xanthomatous degeneration observed in the frozen sections was mistaken for carcinoma and a radical mastectomy performed. The frequency of mistaken diagnosis is still more apparent in Menville's³ series, necrosis of fat having been mistaken for cancer in 20 per cent of the cases and xanthomatous degeneration in an additional 11 per cent. These few but significant figures call for new endeavor in the understanding of the pathologic involvements of the fatty framework of the breast. One primary liposarcoma and 11 new growths of fat tissue found among 274 consecutive mammary tumors removed at operation are presented here. The variety of patterns exhibited by the new growths seemed worthy of comment and an attempt at orderly classification of the growths.

HISTORICAL REVIEW

Fatty tumors of the breast are rare and have interested few writers. The first specimen properly recorded is that now in the Gordon Museum of Guy's Hospital and referred to by Holmes and Hulke⁴ in their "System of Surgery." It consisted of "several pounds of fat which had been growing in the site of the mammary gland for 58 years." The patient died in 1860, at the age of 87 years, having been troubled only by the bulk of the pendulous tumor, which measured 23 inches (58.5 cm.) in circumference. This tumor is representative

From the Department of Pathology, Framingham Union Hospital.

This study was aided by the Lydia Raymond Research and Publication Fund of the Framingham Union Hospital.

1. Haagensen, C. D.: *Am. J. Cancer* **16**:1077, 1932.

2. Keynes, cited by Haagensen.¹

3. Menville, J. G.: *Am. J. Cancer* **24**:797, 1935.

4. Holmes, T., and Hulke, J. W.: *A System of Surgery*, London, Smith, Elder & Co., 1883.

of the usually slow growth of fatty tumors of the breast, but rapid growth of one of these tumors is occasionally noted.

Adair, Pack and Farrior⁵ reported 134 cases of lipoma, in 15 of which the tumor occurred in the breast in a location which made clinical diagnosis difficult. In most of these cases it was retromammary, difficult to palpate and, owing to its elasticity, it often simulated the consistency of a deep cyst. When localized in the mammary fold it caused dimpling where it was attached to the skin, similar to that seen in the case of a sweat gland carcinoma. When in the axilla, it could not always be differentiated from aberrant breast tissue or from cyst of a large apocrine sweat gland.

Among the 622 cases of lipid tumor reviewed by Geschickter⁶ there were 39 in which the breast was involved, in 3 by liposarcoma and in 36 by lipoma. In 5 of the 39 cases, either both breasts were affected, or multiple fatty nodules were present in the same breast—a multiplicity of lesions that obviously might lead to a false impression of cystic disease.

Among the 58 cases of conditions affecting the fatty framework of the breast reported by Menville there were 9 cases of xanthomatous growth, 25 cases of fat necrosis and 24 cases of lipoma. Pain was present in 6.5 per cent of the cases and retraction of skin in 4.4 per cent—data which emphasize the necessity of microscopic study of biopsys material.

There were 27 cases of lipoma (in 1 of which the patient was a male) in de Chohnoky's⁷ series. These comprised 3.7 per cent of all cases of simple tumor of the mammary gland and 1 per cent of all pathologic conditions of the breast. These figures correspond closely with the 2 per cent of lipid tumors of the breast reported by Kleinschmidt.⁸

That fatty tumors of the breast are uncommon enough to warrant description even of individual specimens is shown by the recent reports of Spalding⁹ and Halpert and Young.¹⁰ Spalding recognized among his 3 cases two different types, one consisting of fatty tissue only and the other of a combination of fatty tissue and glandular epithelium, which were growing together in a coordinate fashion to form a well differentiated adenolipoma. This subdivision was not accepted by Halpert and Young. Any new growth of fat tissue, either localized or diffuse, centering around the epithelial structures of the mammary

5. Adair, F. E.; Pack, G. T., and Farrior, J. H.: *Am. J. Cancer* **16**:1104, 1932.

6. Geschickter, C. F.: *Am. J. Cancer* **21**:617, 1934.

7. de Chohnoky, T.: *Arch. Surg.* **38**:79, 1939.

8. Kleinschmidt, O.: *Chirurg* **3**:297, 1931.

9. Spalding, J. E.: *Guy's Hosp. Rep.* **24-25**:80, 1945-1946.

10. Halpert, B., and Young, M. O.: *Arch. Path.* **42**:641, 1946.

glands, they ascribed to lipomatosis. The term "mammary lipoma" they reserved for the growths characterized by a focal aggregation of well differentiated fat cells delimited by a capsule and located within the mammary gland proper.

MATERIAL AND CLINICAL DATA

Among the 274 conditions of the breast requiring surgical intervention at the Framingham Union Hospital during the last ten years, twelve were classified as fat tissue new growths, one of which was a liposarcoma. Thus 4.6 per cent were lipid growths, including simple and cancerous forms, a figure which is slightly higher than that reported by de Cholnoky⁷ and Kleinschmidt.⁸ Simple fat tissue growths made up 5.5 per cent of conditions not requiring mastectomy. The only liposarcoma represented 1.5 per cent of all cancers, which included sixty-three carcinomas and one fibrosarcoma.

Of the 12 fatty tumors of this series, 11 were in females and 1 in a male, a distribution already shown by de Cholnoky⁷ and by Holland.¹¹ The greatest incidence was in the fourth and fifth decades of life, but all age groups were represented, the youngest patient being a woman of 25 and the eldest a woman of 75. None of these tumors were found to have occurred before the age of puberty. In 4 instances the marital status was not mentioned; 2 of the patients were single, and all others had one or more children. Three of the patients were obese and the others of normal habitus.

Only in 2 cases was there a possible relation between the fatty new growth and previous trauma. One case was that of a 49 year old woman whose breast was struck twice, the first time eighteen months and the second time six months prior to the development of the mammary tumor, which occurred at the site of the trauma. In this as in other similar reported cases it was not possible to establish a causative relation between the trauma and the tumor. In the other case, that of a 61 year old woman, a lipoma developed beneath the scar resulting from a radical mastectomy performed for cancer four years before. The fatty mass was excised and found to be noncapsulated. The growth slowly recurred, and three years later the newly formed mass was removed; microscopically, its structure was unchanged.

In 9 of the 12 cases of fatty tumor of the breast the fat tissue growth represented the only pathologic condition that had been seen in the breast. A case in which a previous cancer had been removed has already been mentioned; in another case a fibroadenoma had been removed some time before from the opposite breast, and in a third a fibroadenoma had been excised from the same breast four months before the fatty tumor made its appearance. New growths elsewhere in the body included a cervical polyp in one case and multiple uterine leiomyoma in another.

In none of the cases of simple lipoma was the tumor fixed to the skin or to deeper structures. There was no retraction of the nipple, duplication, local redness or increased heat or palpable axillary lymph nodes. Localized tenderness was present in 10 cases. In 2 cases the preoperative diagnosis was fibroadenoma and in 8 cases cystic mastitis.

The apparent duration of the simple tumors varied from three weeks to five years. Even considering the fact that a tumor may remain unnoticed for a long period, only in 3 instances did one gain the impression that the tumor had rapidly

11. Holland, T. E.: *Canad. M. A. J.* **32**:74, 1935.

increased in size; in the majority of cases the growth of the tumor was slow, with long periods in which it was stationary. In the case of the liposarcoma the patient asked for medical advice one month after she had first noticed the "lump."

LOCALIZATION AND GROSS CHARACTERISTICS

In 5 cases the site of the growth was the right breast; in 7, the left, including the 2 instances with multiplicity of growths. The upper outer quadrant appeared to be the most frequent site (8 cases), followed in order by the upper inner quadrant (3 cases), the fold (1 case) and the areolar edge (1 case). In 1 instance the tumor was retromammary, a localization that is generally considered commoner than the intraglandular one. The patient was a 43 year old woman. The growth, the size of a small cherry at the time it was noticed five years previously, grew slowly, reaching the size of a grapefruit at the time of removal. Roentgenograms of the breast showed an encapsulated mass of uniform density which displaced and compressed the glandular tissue to a thin crescent. A faint shadow suggested that the pectoral muscles overlay the tumor. At operation the tumor was found to be a well encapsulated lipoma, 17 by 13 by 10 cm., located underneath the pectoralis major muscle.

Multiplicity of growth was observed in the breast in 2 cases. In one case a tumor was located at the fold close to the axilla and another in the upper outer quadrant. In the other case two tumors were embedded in the upper outer quadrant.

What was interpreted as a recurrence occurred in a single instance, that of a 61 year old woman previously submitted to mastectomy for carcinoma. Whether this case should be considered among those of mammary lipoma is questionable as it might find better classification among those of subcutaneous lipoma of the mammary region.

The cases of fatty new growth included many varieties of form, size and distribution. In some the tumor was nodular and encapsulated, and in others diffuse deposits involved areas ranging from 1 cm. in diameter to virtually the entire breast. In 8 cases the tumor was encapsulated, and in 6 it was not encapsulated. In one of the 2 cases with multiplicity of growth the breast contained two masses, both of which were nodular and encapsulated. In the other case the breast also contained two masses, both occurring as diffuse fatty deposits. The size ranged from 1.2 to 17 cm. in diameter. The size of the tumor usually varied with its duration, but no definite relation could be found between existence and size.

The gross examination usually showed these tumors to be lobulated, pale yellow or golden yellow, and soft in consistency. Fibrous septums were evident both externally and on the cut section. The size of

the lobules and the thickness of the interlacing fibrous bands varied. Differences in color and in the size of the lobules were often distinct as these growths were compared with the normal surrounding fat.

MICROSCOPIC PATTERNS

As for the intimate structure, the fourteen lipid tumors of this series were classified as follows:

Mature and Well Differentiated Fat Tissue (5 cases).—This was the most frequent pattern in this series. The proliferated fat tissue was the same as normal fat tissue and failed to show increase in cellularity pointing to active potentiality of growth (fig. 1). Tumors of this type would be expected to give a history of slow growth. However, the structure of the most rapidly growing tumor of this series was of this type. Sections taken from many areas of the tumor failed to show foci of cellularity which could account for the rapid growth.

Mature Fat Tissue with Foci of Cellularity (2 cases).—A number of authors have ventured the opinion that recurrences of supposedly simple fatty lipomas are due to the presence of immature cells missed through incomplete histologic study. It is common experience, however, that entirely mature cell growths, arousing no suspicion of potential cancer from the histologic standpoint, may later be the site of repeated recurrences. It is also well known that fatty growths containing immature mesenchymal cells giving a false impression of progressive potentialities of growth frequently fail to recur. This fact suggests that multicentric foci of cellularity represent the actual matrix of new fat cells which, unless accompanied by demonstrable cellular anaplasia and evidence of abnormal mitoses, does not indicate cancer. The 2 tumors of this series in which multicentric foci of cellularity were found were both rather small, one encapsulated. Neither has recurred, one and three years, respectively, after removal.

The cells making up the foci included elements of lymphocytoid character (interpreted as histiocytes) and cells of a larger type, resembling a plasma cell which, as pointed out elsewhere,¹² possibly represents the forerunner of the larger foam cell (fig. 2). The intimate admixture, in these foci, of fibroblasts, histiocytes and lipid cells in various stages of development again raises the question of the genetic relationship of these different cell types.

Figure 3 illustrates a close arrangement of lipoblasts. Whether or not the adipose cells are capable of multiplying once they have

12. Tedeschi, C. G.: Experimental Embryonal Cell Sarcoma, Arch. Path., to be published.

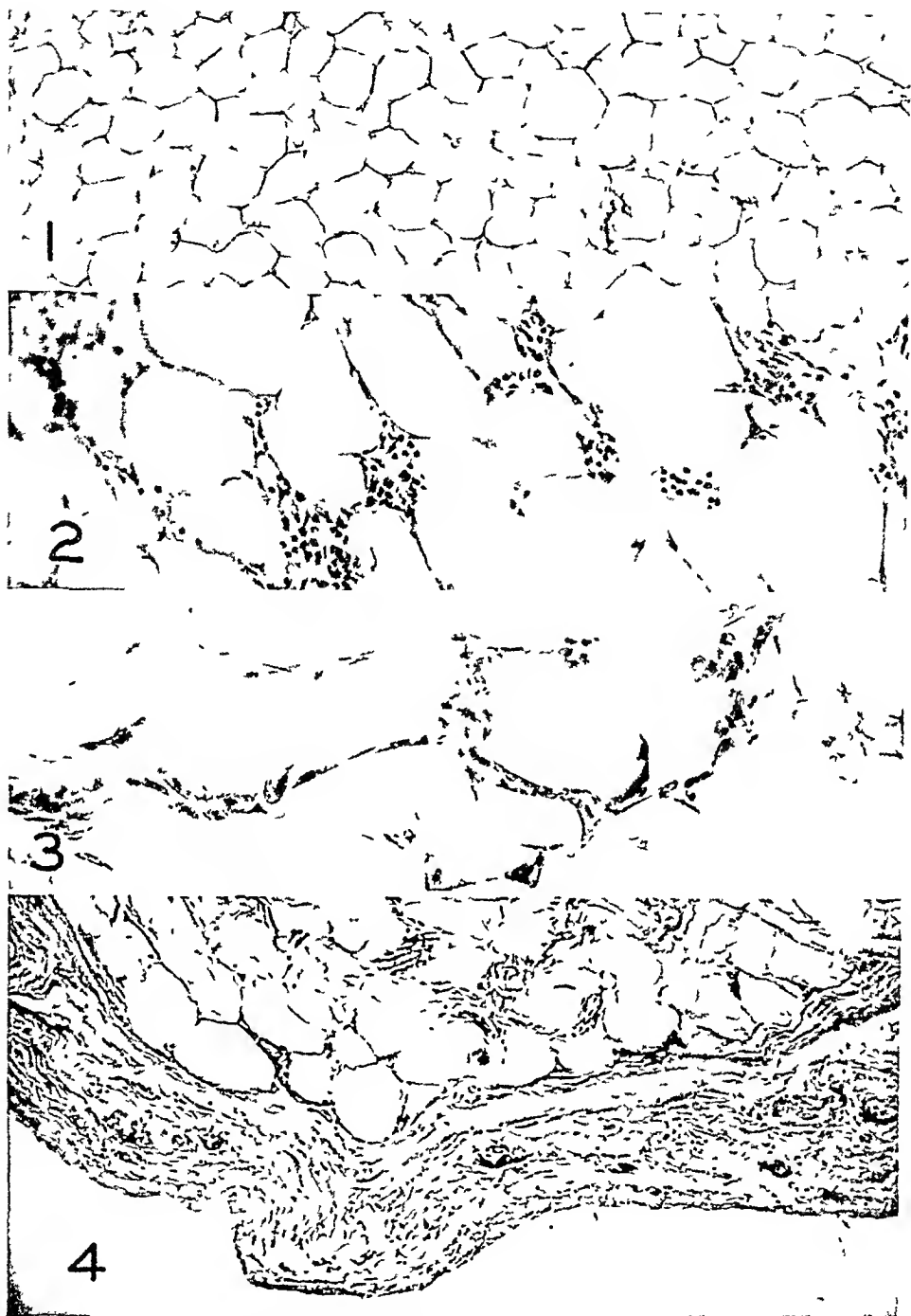


Fig. 1.—Mature, well differentiated lipoma of retromammary localization, growing to the size of a large grapefruit in a five year period. Zeiss lens, ocular 5, objective 10.

Fig. 2.—Encapsulated, slow growing lipoma (2 cm. in diameter) showing foci of cellularity, most numerous at the capsular boundary. No recurrence after three years. Zeiss lens, ocular 10, objective 10.

Fig. 3.—Foci of lipocytic proliferation in a diffuse and well differentiated lipomatous growth. Zeiss lens, ocular 5, objective 40.

Fig. 4.—Collagen formation and marginal sclerosis in a capsulated, mature lipoma. Zeiss lens, ocular 7, objective 10

reached their full maturity (Kölliker¹³) is still a matter of controversy. This possibility was denied by Wassermann,¹⁴ who expressed the opinion that proliferation of fat cells occurs through a process of dedifferentiation by which the adult cell reverts to the original pluripotent reticulum cell that is capable of giving rise to new fat cells. In the absence of any other cellular type and of any transitional pattern which might be interpreted as evidence of cellular differentiation or dedifferentiation, foci of lipoblastic cells such as that shown here might indicate the possibility that the mature fat cell is, under certain circumstances, capable of giving rise to new fat cells.

Involucional Changes in Well Differentiated Fat Tissue (2 cases).—According to the traditional description, the adipose tissue of the adult human being consists of large cells containing fat in the form of single globules which are encircled by a thin envelope of protoplasm. The presiding nucleus, dislocated from the center of the cell to the periphery, gives rise to the signet ring cell, so characteristic of this type of tissue.

A new conception of the structure of the fat cell was introduced some years ago by Marchand¹⁵ and Policard.¹⁶ Marchand, in his study on the transplant of adipose tissue, noted that the cuticle of the fat cell was not of a plasmatic nature and compared it to the sarcolemma of striated muscle. Policard attempted to demonstrate that the membrane of the fat cell was not of ectoplasmic origin but was made up of the collagenous fibers of the fat tissue stroma, against which the fat globule lay directly, without the interposition of any other structure. The fat particles floating in the blood stream would therefore enter the cell by a simple physical process conditioned to the colloidal status of the cell. It is now generally accepted (Schaffer¹⁷) that the wall of the fat cell can be divided into a thin inner protoplasmatic layer enclosing the nucleus at one point of the circumference, and an outer membrane of reticular fibers, the thickness of which depends on the amount of pressure that may be exerted on the cells. Favilli¹⁸ and Volterra¹⁹ have given excellent illustrations of the interlacing of argentaffin fibers enveloping the fat cell. According to Nageotte and Guyon,²⁰ a thick network of interlacing precolla-

13. Kölliker, A.: *Anat. Anz.* **1**:206, 1886.

14. Kassermann, F.: *Ztschr. f. Zellforsch. u. mikr. Anat.* **3**:235, 1926.

15. Marchand, F.: *Beitr. z. path. Anat. u. z. allg. Path.* **61**:1, 1920.

16. Policard, A.: *Compt. rend. Soc. de biol.* **87**:944, 1922.

17. Schaffer, J., in von Möllendorf, W.: *Handbuch der mikroskopischen Anatomie des Menschen*, Berlin, Julius Springer, 1930, p. 74.

18. Favilli, G.: *Sperimentale, Arch. di biol.* **87**:629, 1928.

19. Volterra, M.: *Sperimentale, Arch. di biol.* **81**:319, 1927.

20. Nageotte, J., and Guyon, L.: *Compt. rend. Soc. de biol.* **88**:1288, 1923.

genous fibers, branching and anastomosing on one another, gives rise to a trabeculated structure, in the meshes of which lie the fat cell, "like a balloon suspended in its net."

Under normal circumstances this meshwork cannot be seen unless special stains are applied. It becomes apparent, however, on the formation of collagen in the argentaffin reticulum, an occurrence the significance of which has been little investigated. Thickening of the mesenchymal involucre of the fat cell of a mild degree was seen rather frequently in the fatty new growths of this series. In 2 cases, however, the degree of sclerosis was so striking that a word of comment cannot be amiss. In one case, as represented in figure 4, the process was most apparent at the periphery of the growth (marginal sclerosis), while in the other the process was present throughout the fatty nodule (fig. 5). I would interpret this productive fibrosis as an involutional phenomenon.

Mature Fat Tissue and Glandular Tissue (3 cases).—It has been emphasized many times that the periductal fibrous tissue of the breast must be considered as an intrinsic part of the secretory apparatus. It responds with the glandular structures to ovarian stimulation, and it shares with them practically all the dysplastic mammary states. The intralobular connective tissue which surrounds the secreting elements is, in general, much more cellular than the interlobular connective tissue and contains practically no fat. Since juxtaposition of mammary epithelium and fat does not occur in the breast, as a rule, the combination of epithelial structures and fat tissue in 3 of our cases is unusual. In 2 cases the new growth was nodular and encapsulated and consisted of fatty tissue in which epithelial ducts were sparsely scattered. The fat cells were predominant and lay in direct contact with the ducts (fig. 6). The epithelium lining the ducts was low cuboidal or distorted by reciprocal compression and filled the lumens almost completely. No glandular acini could be recognized in either one of the two new growths. The patterns in these two tumors compare exactly with Spalding's⁹ description of a tumor classified as adenolipoma. He assumed that the tumor had arisen within the mammary lobule by proliferation of the epithelial and connective tissue cells, the latter differentiating into fat cells instead of the usual connective tissue cells. This possibility cannot be denied, but it is more logical to interpret the combination as the result of the revival of embryonal mammary elements segregated into the breast during development. The following points favor this view: (a) the presence of a capsular boundary, (b) the absence of glandular acini and (c), most important, the direct juxtaposition of mammary epi-

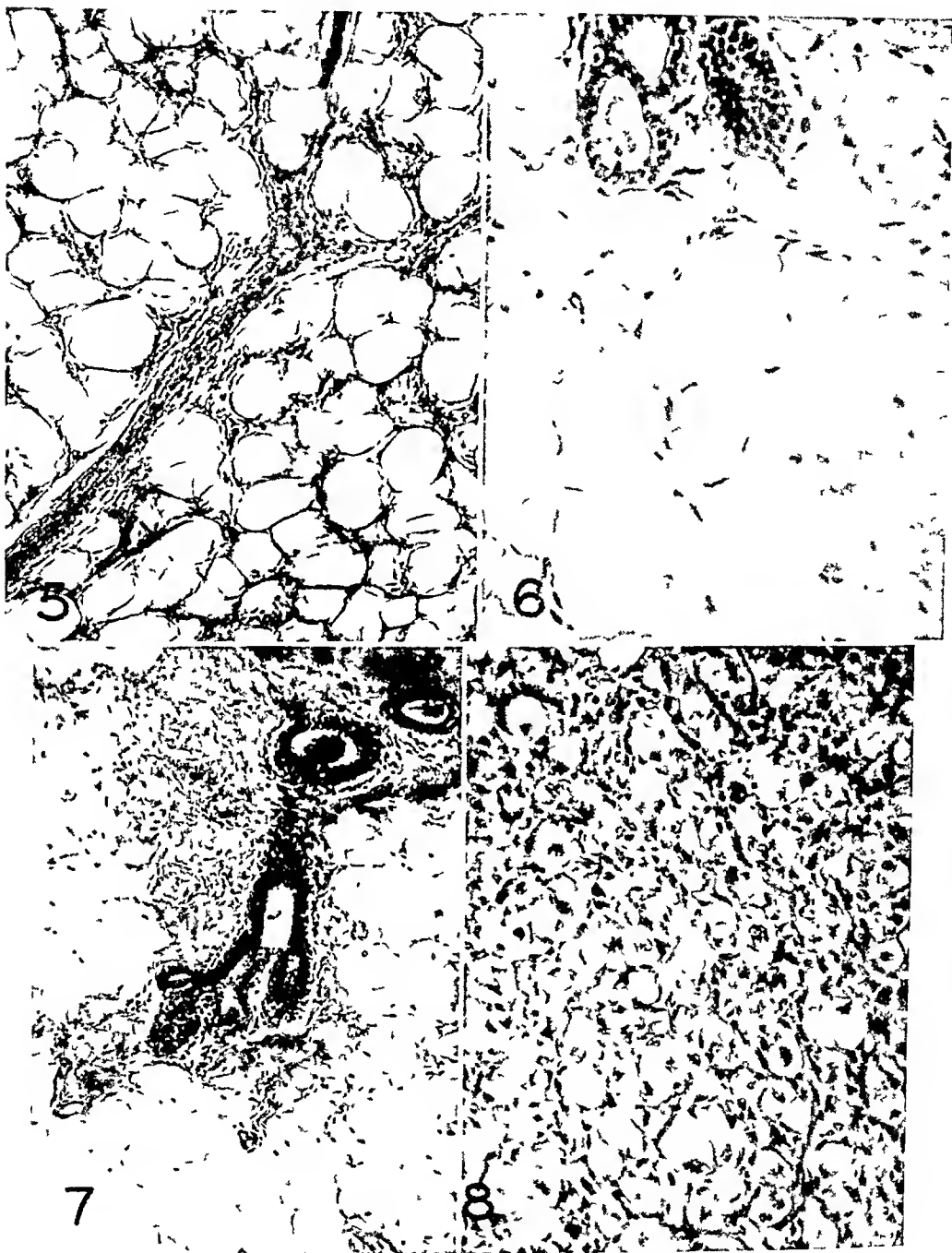


Fig 5—Thickening of the argentaffin reticulum and collagen formation in a diffuse lipomatous growth. Zeiss lens, ocular 7, objective 10.

Fig. 6—Fatty cells lying in direct contact with epithelial ducts. True adenolipoma. Zeiss lens, ocular 10, objective 10.

Fig. 7.—Overgrown fat tissue infiltrating and displacing the tissue proper of the breast and giving rise to an adenolipomatous pattern. Zeiss lens, ocular 7, objective 10.

Fig 8—Liposarcoma, mature cell type. Zeiss lens, ocular 5, objective 4.

thelium and fat, a condition which seems to be found normally only in the embryonal breast (Hamilton, Boyd and Mossman²¹).

Spalding's interpretation might apply, however, to the third case of this group, in which the tumor was characterized by the presence of fat and glandular elements. In this case, however, the new growth was not encapsulated, and between the duct walls and the fat tissue there was a thick layer of connective tissue stroma which was more dense and less cellular than the normal intralobular connective tissue (fig. 7). Glandular acini were also recognizable, and since the epithelial structures were not evenly distributed the resulting pattern may have been brought about by overgrown fat tissue infiltrating and displacing the tissue proper of the breast rather than by intralobular connective tissue cells differentiating into fat tissue cells.

Mature Fat Tissue and Fibrous Connective Tissue (1 case).—This combination was found in but 1 case. The tumor was a small encapsulated nodule; the outer aspect was fatty, whereas the central area was composed of firm, pale gray and glistening tissue from which thin bands radiated in various directions. Except for the capsule, the gross appearance was similar to that of a scirrhus carcinoma. Microscopically, both fat and fibrous tissue were well differentiated, and there were no cellular patterns pointing to a transitional developmental stage. The neoplastic combination of the two tissues might be interpreted as an expression of the multiple developmental potentialities of the undifferentiated mesenchymal cell of the fat lobule.

Liposarcoma (1 case).—Embryologically lipoblasts are found in either of the following forms: Cells which are round or polyhedral and since early development have been grouped together in gland-like lobules of a moruloid structure; or widely spaced stellate or spindle-shaped cells which gradually become rounded and assume characteristics of fat cells as droplets of lipid material accumulate within their cytoplasm and which are embedded in a highly vascularized mucoid mesenchyma. These two embryologic forms are reproduced with great exactness by the cancerous lipoblastic tumors. Those which reproduce the embryonal mesenchymal tissue are generally myxomatous and include a fibrosarcomatous tissue mixed with lipoblasts. The other shows rounded or polygonal lipoblasts of varying sizes and an admixture of mature fat cells and of fat-laden phagocytes without any myxomatous or fibroblastic tissue.

The latter was the pattern shown by the only cancerous lipoblastic tumor included in this series. The patient was a 75 year old woman who had first noticed the mass one month previously. At the time

21. Hamilton, J. J.; Boyd, J. D., and Mossman, H. W.: Human Embryology, Baltimore, Williams & Wilkins Company, 1945.

of operation the tumor was 6 cm. in diameter. Almost all the axillary lymph nodes showed metastatic involvement which exactly reproduced the cellular and structural characteristics of the primary tumor.

In this case the differential diagnosis centers primarily on the xanthoma group. According to Haagensen's¹ classification, xanthoma falls into one or the other of the following two main categories: (a) primary xanthoma (xanthosarcoma of the English and Germans, *xanthom en tumeurs* of the French), which is interpreted as a local manifestation of the syndrome of xanthomatosis; (b) secondary xanthoma (or better, pseudoxanthoma, Aschoff²²), a retrogressive change likely to occur in any inflammatory or neoplastic tissue. The fundamental distinction between the two groups is made on histologic structure. Primary xanthoma is made wholly of xanthoma cells and can occur in any part of the body, including the breast.²³ Pseudoxanthoma contains not only xanthoma cells but also inflammatory cells if arising or growing in inflammatory tissue or among tumor cells. The absence of any other type of neoplastic structure or of a primary inflammatory process rules out in my case xanthomatous degeneration occurring in inflammatory or neoplastic tissue. As for primary xanthoma, the cells of the tumor with their round or polyhedral shape, round nucleus and lightly stained foamy cytoplasm closely simulated xanthoma cells under the lower power of the microscope, but they were larger than the usual xanthoma cells, mature fat cells were present in abundance and no doubly refractive crystals of cholesterol esters could be recognized, either free or intracellular (fig. 8).

SUMMARY AND CONCLUSIONS

The simplest way to classify the fatty tumors of the breast region on topographic and gross criteria seems to be to divide them into four broad groups on the basis of whether the tumor is provided with a capsule or is free from any capsular boundary, or whether it has arisen in the fatty framework of the breast itself (intramammary lipoma) or in the retromammary or the subcutaneous fat and has clinically involved the breast (paramammary lipoma).

As for the microscopic structure, the first main subdivision must be traced between the growths consisting of fatty tissue only and those in which other tissues are present in the tumor.

In the group of the pure fatty growths, the mature and well differentiated type, the sclerosing or involutional type and the one provided with multicentric foci of cellularity deserve recognition not

22. Aschoff, L.: *Pathologische Anatomie*, Jena, Gustav Fischer, 1923, p. 997.

23. Cheatele, G. L., and Cutler, M.: *Tumors of the Breast*, Philadelphia, J. B. Lippincott Company, 1932, p. 307. Haagensen,¹

only for their distinct structural pattern but for the justified expectancy of a different potentiality of growth, that is to say, for the prospect of a different clinical outcome.

In the group of the fatty tumors in which other tissues enter into the composition, a combination of fatty tissue and of fibrous connective tissue, on one hand, and of fatty tissue and of epithelial glandular elements, on the other, exemplify the two types of homologous and heterologous mixed fatty tumor.

The homologous type is interpreted as an expression of the multiple developmental potentialities of the undifferentiated mesenchymal cell of the fat lobule.

Since juxtaposition of mammary epithelium and fat does not, as a rule, occur in the mature breast but can be found in the embryonal breast, a revival of embryonal mammary elements segregated into the breast tissue during development is the hypothesis proposed to explain the coordinate proliferation of fat tissue and of glandular elements. This suggestion, of course, does not rule out the possibility that a mixed fatty and glandular tumor may have resulted because intralobular connective tissue cells differentiated into fat tissue cells. In both eventualities the ensuing pattern is that of true adenolipoma. This label obviously does not apply to tumors in which a combination of glandular elements and fatty tissue has been brought about because the tissue proper of the breast was infiltrated and displaced by overgrown fat tissue, as illustrated in one of my cases.

The subdivision generally accepted for liposarcoma, namely an embryonal type and a mature cell type, stands also for the cancerous growths arising in the fatty framework of the breast. The only cancerous lipoblastic tumor included in this series exemplified the mature cell type.

ABSENCE OF RENAL LESIONS IN RATS RECEIVING A SYNTHETIC DIET LOW IN PROTEIN

ELIZABETH LOWENHAUPT, M.D.
SAN FRANCISCO

IN AN interesting physiologic and pathologic study of rats receiving a low protein, natural diet with either carrots or turnips as the primary constituent, Dicker, Heller and Hewer¹ observed histologic renal lesions of some degree in 67 of 84 rats, or 80 per cent. The lesion consisted of calcified renal tubular casts with a surrounding foreign body reaction, similar to those seen in chronic alkalosis² and in dietary chloride deficiency producing chronic alkalosis.³ For this reason it seems of interest to determine whether low protein intake itself produces this renal lesion or whether some other property of the natural diet is to be considered responsible.

METHODS

The following diet was employed: casein 1 per cent, sucrose 75 per cent, vegetable oil 17 per cent, salt mixture 4 per cent, cod liver oil 2 per cent and wheat germ oil 1 per cent. To each kilogram of diet the following supplements were added: thiamine hydrochloride, inositol and para-aminobenzoic acid, 160 mg. of each; calcium pantothenate, riboflavin and pyridoxine, 80 mg. of each; nicotinic acid 500 mg. and choline chloride 2.0 Gm. Male rats were used, 5 varying in weight from 325 to 360 Gm. and 5 from 140 to 180 Gm. The animals were maintained on this diet until each had lost approximately one third of its original body weight, a decline requiring approximately eight weeks in the first group and five weeks in the second. At this time rats were killed and tissues fixed for histologic examination.

RESULTS

None of the rats showed the renal tubular obstructive lesion described previously.¹ One of the larger rats showed extreme fatty infiltration of the liver with early cirrhosis of the type described by György and Goldblatt⁴ and by Webster.⁵

From the Division of Pathology, University of California.

1. Dicker, S. E.; Heller, H., and Hewer, T. F.: *Brit. J. Exper. Path.* **27**:158, 1946.

2. Cooke, A. M.: *Quart. J. Med.* **2**:539, 1933.

3. Lowenhaupt, E., and Greenberg, D. M.: *Arch. Path.* **42**:49, 1946.

4. György, P., and Goldblatt, H.: *J. Exper. Med.* **75**:355, 1942.

5. Webster, G. T.: *J. Clin. Investigation* **21**:385, 1942.

COMMENT

The duration of the experiment and the loss of weight on this synthetic diet are approximately equal to those on the natural diet.¹ Although the protein of the latter diet cannot be accurately computed, that of the former produces a similar biologic response. The absence of renal tubular lesions would suggest that lack of protein alone is not the responsible factor.

The original authors¹ excluded choline deficiency and high serine dietary content as responsible agents, mentioning the similar lesions produced in uric acid and in phosphate nephritis. However, they failed to consider the possible contribution of the high content of carrot or of turnip (85 per cent). Of all usual vegetables,⁶ both carrots and turnips are among those that are highest in residual alkaline elements: carrots with 14 cc., turnips with 12 cc. and potatoes next with 9 cc. of normal alkali per hundred grams. Both carrots and turnips are high in calcium and in phosphates.⁶ Thus it seems quite likely that this diet might produce a chronically alkaline urine containing large quantities of calcium and phosphates. This is the ideal condition for the precipitation of calcium phosphate casts in the ascending loops of Henle and in the distal convoluted tubules as discussed in the case of chloride deficiency of the rat.³ This is likewise observed in chronic alkalosis,² and, just as in both of those conditions, the lesion consists first in the presence of precipitated calcium with a consequent surrounding foreign body reaction. Inability to acidify the urine might be increased by the physiologic damage of low protein.¹

The low incidence of hepatic lesions is to be explained by the acuteness and the short duration of the deficiency in contrast to the longer survival on a diet higher in protein.⁴

SUMMARY

It is concluded that low protein intake is not responsible for the renal tubular lesion occurring in rats fed a low protein, carrot and turnip diet. Chronically alkaline urine containing large amounts of calcium and phosphates is suggested as the basis for the precipitation of calcium in the tubules, with a foreign body reaction occurring about the cast. An identical lesion is described in chronic alkalosis and is produced in the rat by a chloride-deficient diet.

6. Bowes, A. deP., and Church, C. F.: *Food Values of Portions Commonly Used*, ed. 6, Philadelphia, Anna de Plantar Bowes, 1946. Sherman, H. C., and Lanford, C. S.: *Essentials of Nutrition*, New York, The Macmillan Company, 1940.

Books Received

BRONCHIOGENIC CARCINOMA AND ADENOMA. WITH A CHAPTER ON MEDIASTINAL TUMORS. By B. M. Fried, M.D., associate attending physician of Montefiore Hospital for Chronic Diseases, New York. Pp. 306, with 118 illustrations. Price \$6. Baltimore: Williams & Wilkins Company, 1948.

This book consists of 306 pages and considers the subject of bronchogenic carcinoma in all its aspects. Much space (106 pages) is devoted to incidence, pathologic anatomy, etiologic factors and metastases. These features are presented in a commendable manner, and this part of the book seems a good source for reference purposes.

The chapters devoted to clinical manifestations and diagnosis present the material well and bring out the most recent developments. The more important features of the condition are stressed, and much clinical material is used to emphasize the various aspects of the disease.

The chapter on treatment is quite inadequate and at times misleading. There is a general tendency to overemphasize the value of roentgen therapy. Much of the reference material used in the discussion of surgical management of the lesion is old and outdated. Few of the advances described in recent publications are mentioned. Thus, there is ground for real criticism of an otherwise well prepared book concerning this most important problem.

The chapter on adenoma is satisfying for such a controversial subject. The chapter on mediastinal lesions is sketchy. In the opinion of the reviewer this material would better have been omitted except as a subject for discussion in the differential diagnosis of primary tumors of the lung.

HISTOPATHOLOGY OF THE PERIPHERAL AND CENTRAL NERVOUS SYSTEMS. By George B. Hassin, M.D., emeritus professor of neurology at the University of Illinois College of Medicine, Chicago. Third edition (revised and enlarged). Pp. 612, with 325 illustrations. Price \$8.50. Privately printed, 1948.

The first edition appeared in 1933, the second in 1940. The present edition includes the most recent advances in its field. Chapters on catabolic diseases, trauma of the cauda equina and the histology of spina bifida are added; also new illustrations; and the bibliographies and the index are enlarged. There are five parts: general considerations, diseases of peripheral nerves and muscles, diseases of the spinal cord, diseases of the brain, staining methods. The illustrations, all black and white, are well done. Hassin's book continues to be a good guide to the study of the microscopic morphology of diseases of the nervous system.

AGING PROCESSES IN THE OVARIES OF MICE BELONGING TO STRAINS DIFFERING IN THE INCIDENCE OF MAMMARY CARCINOMA

LEO LOEB, M.D., D.Sc.

ST. LOUIS

I. THE FATE OF FOLLICLES AND CORPORA LUTEA

THE PRESENT investigations on aging processes in the ovaries of various strains of mice represent a part of a larger series in which the changes taking place in old age in the ovaries, mammary glands, vagina, uterus and the thyroid and adrenal glands were studied mainly in mice but to some extent also in guinea pigs. In addition the skeletal system has been investigated from this point of view by Martin and Ruth Silberberg.

MATERIAL

We distinguish four periods in the life of the mouse as follows: period 1 (from birth up to 9 months of age), period 2 (from 10 to 14 months), period 3 (from 15 to 19 months) and period 4 (20 months and older).

Mice of the various strains were distributed in these four periods as follows:

PERIOD 1.—Strain C: 20 nonbreeding and 4 breeding mice. Strain AKA: 34 nonbreeding and 2 breeding mice. Strain CBA: 17 nonbreeding mice; 1 breeding mouse. Strain C57 black: 53 nonbreeding and 5 breeding mice. Strain Old Buffalo: 2 nonbreeding mice. Strain New Buffalo: 3 nonbreeding mice. Strain C3H: 19 nonbreeding and 7 breeding mice. Strain D: 20 nonbreeding and 6 breeding mice. Strain A: 45 nonbreeding and 3 breeding mice. In period 1 the total numbers were 213 nonbreeding and 28 breeding mice, altogether 241 mice.

PERIOD 2.—Strain C: 3 nonbreeding and 4 breeding mice. Strain AKA: 11 nonbreeding and 2 breeding mice. Strain CBA: 9 nonbreeding and 5 breeding mice. Strain C57 black: 28 nonbreeding and 14 breeding mice. Strain Old Buffalo: 17 nonbreeding and 2 breeding mice. Strain New Buffalo: 2 nonbreeding and 10 breeding mice. Strain C3H: 17 nonbreeding and 14 breeding mice. Strain D: 49 nonbreeding and 14 breeding mice. Strain A: 44 nonbreeding and 29 breeding mice. In period 2 the total numbers were 180 nonbreeding and 94 breeding mice, altogether 274 mice.

From the Laboratory of Research Pathology, Washington University School of Medicine.

The ovaries, on the microscopic examination of which these investigations are based, were collected by H. T. Blumenthal, Marian Moskop Kirtz and V. Suntzeff, in the course of various experiments carried out in this laboratory.

PERIOD 3.—Strain C: 3 breeding mice. Strain AKA: 5 nonbreeding and 3 breeding mice. Strain CBA: 2 nonbreeding and 8 breeding mice. Strain C57 black: 12 nonbreeding and 12 breeding mice. Strain Old Buffalo: 2 nonbreeding and 4 breeding mice. Strain New Buffalo: 14 nonbreeding and 5 breeding mice. Strain C3H: 3 nonbreeding and 4 breeding mice. Strain D: 12 nonbreeding and 4 breeding mice. Strain A: 16 nonbreeding and 10 breeding mice. In period 3 the total numbers were 66 nonbreeding and 53 breeding mice, altogether 119 mice.

PERIOD 4.—Strain AKA: 4 nonbreeding mice, 20 to 23 months of age; 1 breeding mouse, 24 months of age. Strain CBA: 8 nonbreeding mice, 21 to 31 months of age; 6 breeding mice, 20 to 28 months of age. Strain C57 black: 9 nonbreeding mice, 20 to 25½ months of age; 1 breeding mouse, 26 months of age. Strain Old Buffalo: 4 nonbreeding mice, 24 to 29 months of age; 8 breeding mice, 20 to 28 months of age. Strain New Buffalo: 4 nonbreeding mice, 20 to 28 months of age; 2 breeding mice, 23 months of age. Strain C3H: 1 nonbreeding mouse, 20 months of age. Strain D: 4 nonbreeding mice, 20 to 22 months of age; 1 breeding mouse, 22 months of age. Strain A: 7 nonbreeding mice, 20 to 24½ months of age; 1 breeding mouse, 21 months of age. In period 4 the total numbers were 41 nonbreeding and 20 breeding mice, altogether 61 animals.

The numbers of mice of all periods combined were 500 nonbreeding and 195 breeding mice, altogether 695 animals. We designate as breeding mice all those female mice which had one or more litters in their life.

OBSERVATIONS

In this first section I shall study the fate of the follicles and corpora lutea with advancing age. In the second section I shall study the interstitial gland, the ducts developing in the ovary and the changes which they undergo with advancing age. I shall record only the more significant findings in the various strains and refer to some others in the discussion of the observations.

STRAIN C.—*Follicles*.—Period 1. In the center of atretic follicles there were groups of yellow cells, presumably representing modified granulosa cells. During the process of atresia connective tissue cells may penetrate through the membrana granulosa and surround the egg; in 1 instance a granulosa cell seemed to penetrate between the segments of an egg.

Period 2. Large follicles were present in almost all the mice. In one atretic follicle hemorrhage was noted around the egg. Blood vessels could grow into the granulosa of follicles during the process of atresia.

Period 3. Large follicles had developed in all 3 mice.

Corpora Lutea.—Period 1. Only exceptionally hyalinization or gelatinization was seen in a corpus luteum. Degeneration usually occurred by way of vacuolation of the lutein cells and by ingrowth of connective tissue, and following this, the shrunken corpora lutea could be replaced by interstitial gland tissue. This mode of degeneration was the usual one.

Period 2. Vacuolation of the corpora lutea could begin in the center of these structures and progress toward the periphery.

Period 3. In all 3 breeding mice preserved corpora lutea, and in 2 of them even young corpora lutea, were found. Typical hyalinization was lacking, but a softening and solution of the central parts of the corpus luteum could occur, a process which was perhaps related to gelatinization.

STRAIN AKA.—*Follicles*.—Period 1. During atresia the membrana granulosa might become vacuolar or a part of it might become dissolved; connective tissue cells might penetrate through the granulosa to the egg, and in some instances they entered the egg.

Period 2. Some enlarged follicles were seen in 1 mouse. In another mouse one end of the ovary consisted of dilated capillaries.

Period 3. In 1 mouse very large follicles with central hemorrhage were present. In some of the ovaries primordial follicles were noted. In the majority of the ovaries there were full-sized follicles.

Period 4. In the youngest mouse very large follicles were seen, but usually the follicles reached only a small size; then degeneration of the granulosa set in. But also in the oldest mouse very large and hemorrhagic follicles were found.

Corpora Lutea.—Period 1. Some mitoses occurred in luteal cells of young corpora lutea in a mouse 2 months, 6 days of age. After these structures had reached full development, they underwent degenerative vacuolation and then were invaded by connective tissue and also by interstitial gland tissue; in the end shrinking took place. Hyaline changes were seen in the corpora lutea only exceptionally.

Period 2. In 5 mice bluish-staining young, as well as fully mature preserved, corpora lutea were present. But in the majority of these animals there were in addition corpora lutea in various stages of hyalinization. In some ovaries almost all the corpora lutea were hyalinized. Also hyaline arteries were seen. In 1 of the 2 breeding mice there were two hyaline remnants of corpora lutea, and here also the walls of the arteries were largely hyaline.

Period 3. A hyaline artery was seen in the ovary. In 2 breeding mice there were young bluish corpora lutea, in some of which beginning of hyalinization seemed to be noticeable. Older corpora lutea were to a variable degree or even entirely hyalinized. The hyalinization seemed to begin around arteries; there was also hyalin around arteries in various parts of the ovaries. In 1 mouse some corpora lutea underwent vacuolar degeneration.

Period 4. In the majority of these animals there were shrunken corpora lutea, remnants of corpora lutea and hyaline or vacuolar corpora lutea. In 3 younger nonbreeding mice some remnants of vacuolar or shrunken corpora lutea were found; there occurred also hyaline and partly gelatinous corpora lutea. In the single breeding mouse a great part of the ovary consisted of gelatinous corpora lutea in which the nuclei had been lost. Such necrotic gelatinous areas could be surrounded by foreign body giant cells.

STRAIN CBA.—*Follicles*.—Period 1. In the breeding mouse a whole strand of primordial follicles was seen in tissue adjoining the medulla. In atretic follicles the centrally situated cells, probably granulosa cells, could be changed into vacuolar yellow cells and a thin layer of connective tissue could grow to the inner margin of the membrana granulosa lining the central cavity.

Period 2. In two mice some hemorrhage was seen in follicles; in one of these mice a very large follicle was hemorrhagic. In several mice the granulosa of atretic follicles carried yellow pigment.

Period 3. Among the older mice of this period, 2 mice had large follicles, and one of these had also very large follicles. In 3 animals no follicles were seen. Also primordial follicles occurred in this group of mice. Among the younger mice, no follicles or only atretic ones were seen in 2 mice, and in a third one large follicles were lacking.

Period 4. No follicles were found in the 10 oldest mice. Also in the younger mice of this group no large follicles had developed.

Corpora Lutea.—Period 1. Hyalinization was not observed in the corpora lutea of this period.

Period 2. Some young, bluish-staining corpora lutea were noted, but mostly the corpora lutea were older, and red-staining vacuolation, but no hyalinization, appeared in these structures. Some corpora lutea seemed to have been replaced by interstitial gland tissue. When during the early stages of degeneration the circulation was slowed down in a corpus luteum, leukocytes could collect in the central portion of the corpus luteum.

Period 3. Mostly no corpora lutea or only remnants of corpora lutea were found.

Period 4. In 3 nonbreeding mice remnants of corpora lutea or connective tissue-invaded degenerating corpora lutea were present; in 1 nonbreeding mouse red-staining corpora lutea were found; in all the other animals corpora lutea were lacking. There were no hyaline corpora lutea.

STRAIN C57 BLACK.—*Follicles*.—Period 1. The large majority of the mice had large follicles. During atresia the granulosa cells changed into vacuolar yellow cells in some ovaries; it appeared that these yellow cells had phagocytosed red blood corpuscles. Enlarged follicles, some of which were hyperemic or hemorrhagic, occurred in a few mice.

Period 2. In almost all the mice full-sized follicles were present, and in 14 of 42 animals mature or just ruptured follicles were seen. In some of the nonruptured follicles hemorrhages developed, probably having their origin in capillaries growing into the granulosa. Capillaries were seen to invade the granulosa of just ruptured follicles as well as that of nonruptured follicles. The nonruptured follicles could give rise to the formation of pseudo lutein bodies.

Period 3. In a number of mice large follicles were absent; in others, between 15 and 17 months old, mature or just ruptured follicles were seen. In several mice very large, hemorrhagic follicles were noted; hemorrhages occurred also in atretic follicles. Characteristic of strain C57 black mice was the tendency of granulosa cells in degenerating follicles in some animals to form syncytia; at the same time the nuclei could assume a rosette-like arrangement.

Period 4. In 2 mice the ovaries were infiltrated with, and partly replaced by, leukemic lymphocytes. In one half of the remaining mice some follicles reached full development; in the other half the follicles remained small or medium sized. Nonatretic, as well as atretic, follicles could be hemorrhagic, and in atretic hemorrhagic follicles vacuolar granulosa cells seemed to take up blood pigment.

Corpora Lutea.—Period 1. No hyaline corpora lutea were seen.

Period 2. There was no hyalinization. Some pseudo corpora lutea developed after capillaries grew into the membrana granulosa of nonruptured follicles.

Period 3. Among the older mice of this period, between the ages of 17 and 20 months, several animals had good corpora lutea, and in one of them young corpora lutea were seen. In 1 mouse hyalinization of corpora lutea was noted. Also a hyaline artery and a small hyaline area were found in the center of the ovary in 1 mouse. In 2 animals, lacking corpora lutea, hyaline material surrounding capillaries extended from the hilus upward. Hyalinization around vessels could occur probably independently of corpora lutea.

Period 4. There were no preserved corpora lutea during this period, nor were there degenerating vacuolar or hyalinized-gelatinized corpora lutea; but in a

mouse 20½ months of age and weighing 22 Gm. two fairly young corpora lutea with some vacuolation and hemorrhage in the center were seen.

STRAIN OLD BUFFALO.—Follicles.—Period 2. In the large majority of mice large follicles were present, and in 4 animals mature or just ruptured follicles were seen. In some large follicles hemorrhages occurred. In an atretic follicle some granulosa cells moved between the segments of a segmented egg.

Period 3. In the large majority of the animals large follicles were lacking. In a medium-sized follicle there was some hemorrhage.

Period 4. In 5 mice, 26 months of age or older either no follicles were seen or follicles reached at most small to medium size. In mice younger than 26 months, large follicles or even follicles which approached maturity occurred. A primordial follicle was found in the interstitial gland.

Corpora Lutea.—Period 2. Regression took place by shrinking and vacuolation of lutein cells. Only in 1 mouse were hyaline corpora lutea seen, and here hyalinization began around blood vessels. There was also ingrowth of connective tissue in two hyaline corpora lutea.

Period 3. In 1 nonbreeding mouse lutein-like tissue of uncertain nature was seen in which colloid and vacuolar changes took place in some cells. In the 4 breeding mice preserved corpora lutea, and in 1 mouse almost 20 months old even young corpora lutea were present. In the oldest mouse there were some strands of hyaline tissue.

Period 4. Among the 4 nonbreeding mice, varying in age between 24 and 29 months, no corpora lutea were found in 2 animals. In the remaining 2 there were large masses of gelatinous corpora lutea. In 2 breeding mice, 28 months old, there seemed to be preserved corpora lutea. In 2 breeding mice 26 months old, there were gelatinous corpora lutea or corpus luteum-like tissue. In leukemic mice about 20 months of age the greater part of the corpora lutea, including partly hyalinized or gelatinized corpora lutea, had been destroyed by lymphocytes. In the 2 remaining animals, 20 to 21 months old, preserved mature or vacuolar corpora lutea were noted.

STRAIN NEW BUFFALO.—Follicles.—In periods 1 and 2 the follicles reached their full development in most cases; however, in a mouse with low weight, the ovaries were hypotypical.

In period 3 all variations were seen from ovaries with fully developed follicles to ovaries devoid of all follicles or containing only atretic follicles. Some follicles became cystic, and hemorrhages were seen in some very large follicles, but also in other follicles, including atretic ones.

In period 4 conditions were similar to those observed in period 3. Hemorrhages occurred here also, and it seemed that hemorrhages in the follicles could give rise to yellow pigment in the granulosa cells.

Corpora Lutea.—Periods 1 and 2. In some mice regression of corpora lutea took place by vacuolation, but in 4 animals of period 2 hyaline-gelatinous corpora lutea were seen.

Period 3. In about one half of the mice no corpora lutea or only doubtful ones were found; but even in 19 to 20 month old mice new corpora lutea could develop. In a number of mice, especially in the older ones in this period, gelatinization occurred in corpora lutea. In the younger mice corpora lutea in general were better preserved than in the older mice of this period.

Period 4. In mice as old as 28 months, as well as in younger mice, mostly remnants of corpora lutea were found. In some of the animals the corpora lutea

underwent vacuolation, connective tissue ingrowth and shrinking, while in others they underwent hyalinization. Some hyaline clumps which were observed probably represented also remnants of hyaline corpora lutea.

STRAIN C3H.—*Follicles*.—Period 1. In the large majority of mice in this period, and also in period 2, large follicles were found; also mature follicles were seen in some cases. During atresia in periods 1 and 2 the granulosa cells could undergo vacuolation and acquire yellow pigment; also the theca cells could become vacuolated. Connective tissue was seen to invade the membrana granulosa.

Period 2. In atretic follicles both theca and granulosa cells could be vacuolated, but they could be distinguished from each other by the fact that the granulosa cells were the larger. These two tissues could be separated by a hyaline band.

Period 3. In all the mice some large follicles were seen and in 2 mice there were very large or cystic follicles. Hemorrhagic follicles, in which the hemorrhage had destroyed the granulosa, also occurred.

Period 4. In the single mouse examined at this period no large follicles and only a single small follicle were seen.

Corpora Lutea.—Period 1. In the nonbreeding mice corpora lutea in various stages of development were found. Connective tissue penetrated into some of the older corpora lutea which were in process of regression; also remnants of such regressing corpora lutea were seen. In the breeding mice the findings were similar; but there was 1 mouse, 9 months of age and weighing 39 Gm., which showed a peculiar condition. In one ovary there were a number of well developed corpora lutea, but no new corpora lutea were found. In the other ovary there was a blood clot. At one end this ovary was seen to be connected with the fallopian tube. The blood clot was lined with several layers of cells, the nuclei of which were unusually large and contained prominent nucleoli. Some of these cells had two nuclei. These cellular layers around the blood clot represented syncytial formations which were similar to those observed in ovaries of the guinea pig, where in the center of such layers in several instances well preserved embryonal structures were found. Furthermore, in the guinea pig some of the syncytial giant cells penetrated into the surrounding tissue. Similarly in this mouse the corresponding cells migrated into the wall of the fallopian tube, in which a nest of such cells was seen. In addition to these syncytial structures, folds of the germinal epithelium which was connected with the surface of the ovary surrounded the blood clot. This condition might represent either an extrauterine pregnancy in which the fertilized ovum had fixed itself to the fallopian tube or parthenogenetic development of an egg, which would correspond to the findings in the ovaries of guinea pigs. However, there was no pregnancy in the uterus, and it appears more probable, therefore, that in this mouse one has to deal with parthenogenetic development of an egg which attached itself to a place where fimbria and germinal epithelium of the ovary were connected with an abortive extrauterine pregnancy. The blood clot owed its origin presumably to a hemorrhage which was induced by erosion of blood vessels. This resulted when embryonal placental lining cells migrated through the blood vessel walls into neighboring areas. Also in guinea pigs hemorrhages could be caused in this way.

Period 2. Only in some of the nonbreeding mice did hyalinization of the corpora lutea occur; but in these animals this process could progress so far that the greater part of the ovary consisted of gelatinous material, in which vessels with necrotic walls were seen. In only 3 of the 14 breeding mice did hyalinization of the corpora lutea occur, and in 2 of these animals hyaline material surrounded the arteries.

Side by side with the processes of hyalinization there was development of new corpora lutea in some ovaries. In 1 animal regression of a corpus luteum took place by vacuolation.

Period 3. In the oldest of the 3 nonbreeding mice no corpora lutea were seen; in the remaining 2 animals gelatinization of corpora lutea occurred to different degrees. Various adjoining corpora lutea were seen to coalesce, with gelatinization of a great part of the ovary resulting. At the same time new corpora lutea could form, which then proceeded toward maturation. Some hyalinization occurred around small arteries. Some corpora lutea, however, degenerated by vacuolation.

Period 4. In the only mouse present at this period, a nonbreeding animal, 20 months of age and weighing 29 Gm., gelatinized as well as better preserved corpora lutea were seen.

STRAIN D.—Follicles.—Period 1. The membrana granulosa in atretic follicles could be vacuolar, and it contained either yellow pigment or no pigment.

Period 2. Large follicles were seen in the large majority of the animals. In a number of mice mature or recently ruptured follicles were present, and in other animals larger than normal follicles occurred. As usual a great part of the granulosa remained preserved during the early stages of atresia, but it could undergo vacuolation and a layer of connective tissue could cover the inner surface of the granulosa; in 1 animal the ingrowing connective tissue destroyed the eggs in the centers of atretic follicles. In later stages of atresia the greater portion of the granulosa might be destroyed and connective tissue might fill the central cavity.

Period 3. In 9 of the 12 nonbreeding mice no full-sized follicles were seen, and in 2 of these 9 mice follicles were lacking altogether. In 1 mouse a larger than normal follicle was observed, and in another mouse there was blood in the central cavity. In still another animal the greater portion of the ovary had been destroyed by a blood clot. In the breeding mice there were ovaries in which no follicles were preserved or in which follicles were entirely lacking; but in 1 mouse follicles were well developed; also a hemorrhagic follicle was seen in an ovary.

Period 4. No follicles were seen except in 1 nonbreeding mouse, 22 months old, in which some small preserved follicles and a primordial follicle were noted. The presence of follicles in older mice is not, therefore, characteristic of high mammary tumor strains and does not distinguish them from low tumor strains.

Corpora Lutea.—Period 1. In this period and also in period 2 the mice in which corpora lutea were lacking were usually of low weight; this of course does not apply to young mice in which, on account of their age, ovulation had not yet taken place. The corpora lutea which had formed in the other mice were free of hyalinization. Also in the breeding mice hyalinization was lacking.

Period 2. Mitoses appeared in preserved corpora lutea, although rarely. After the stage of maturation has been reached, as a rule processes of hyalinization set in; but in some animals hyalinization was delayed or did not occur; instead, ingrowth of connective tissue and shrinking of the corpora lutea took place. However, vacuolation of the corpora lutea was rare as compared with hyalinization and gelatinization. Hyalinization could also affect the arteries in corpora lutea. Occasionally a deposit of calcium salts was found in gelatinized areas. In 1 animal, in a certain place in the corpus luteum some corpus luteum cells seemed to send pseudopodia out into adjoining gelatinous areas. This picture suggested the beginning of a process of invasion in which the gelatinous material might be substituted by the adjoining preserved corpus luteum tissue. Hyaline rings were seen surrounding capillaries in a corpus luteum. Hyalinization may first appear

around the vessels and then extend to the lutein cells, in which hyaline droplets become visible. Connective tissue and blood vessels also may grow into gelatinized areas. Interstitial glands may take part in the invasion of gelatinized corpora lutea.

Period 3. Hyalinization and gelatinization of corpora lutea were pronounced, and hyalinization seemed to begin in the periphery of the corpora lutea. In some peripheral areas of ovaries and in places underneath the germinal epithelium there could still be seen some better preserved corpus luteum tissue. In the hyalinized and gelatinized tissue some walls of arteries were hyalinized. New corpora lutea still developed. In 5 mice no preserved corpus luteum tissue was found, the ovaries consisting largely of gelatinous material. On the other hand, in 2 mice, 15 and 16 months of age, there were mature or shrunken but not hyalinized or gelatinized corpora lutea, into which connective tissue seemed to have grown. In the 2 oldest mice, 19 months old, among 4 breeders some vacuolated corpora lutea were seen. In mice about 16 months old hyalinization of corpora lutea was lacking; instead there were preserved young corpora lutea.

Period 4. In all the 4 nonbreeding mice, 20 to 22 months of age, small preserved corpora lutea were present besides larger gelatinized corpora lutea or remnants of gelatinized corpora lutea. In 2 the corpus luteum tissue had some tumor-like characteristics; it contained large nuclei and seemed to be slowly growing. In another mouse what appeared as young corpus luteum tissue consisted apparently of coiled ducts, and this tissue likewise had in some respects a tumor-like appearance. Parts of some corpora lutea were preserved, while other parts were hyalinized. In the breeding female there were mainly corpora lutea in different stages of gelatinization, and solution processes also had occurred. In gelatinized corpora lutea there were hyalinized vessel walls in which in places some calcium salts had been deposited.

STRAIN A.—*Follicles*.—Period 1. Only in a small number of mice were large follicles lacking, and these animals had a low weight.

Period 2. Very large and mature follicles occurred similar to those found in period 1. There were in addition the ordinary large follicles. In 4 mice cystically dilated, hemorrhagic follicles were seen.

Period 3. (a) Mice 17 to 19 months of age: There was only 1 animal in which the follicles were not larger than of small to medium size. In several mice very large and even cystic follicles were seen, and in 2 animals some follicles had just ruptured. In some of the mice primordial follicles were present, such as had been observed also in period 1. (b) Mice 15 to 17 months of age: In almost all these mice large follicles were present in addition to very large and cystic, hemorrhagic follicles in some of the mice. In a large follicle in which the membrana granulosa had been lost in the upper segment, the granulosa cells elsewhere had enlarged around the egg; they became vacuolar, and some of them seemed to phagocytose a part of the egg. In several other follicles some granulosa cells seemed to contain phagocytosed material. Other granulosa cells could disintegrate into colloid substance. In some atretic follicles the theca interna enlarged and became vacuolated, while the granulosa cells enlarged and became like corpus luteum cells. Thus pseudo corpora lutea developed.

Period 4. In 3 of 8 mice no full-sized follicles developed, but almost all the animals had some cystic or very large follicles with or without hemorrhagic changes.

Corpora Lutea.—Period 1. Corpora lutea were lacking not only in some young mice, which presumably had not yet ovulated, but also in several older mice of this period, especially if their weight was below 20 Gm. Regression of the corpora

lutea occurred, as a rule, by vacuolation and connective tissue ingrowth. In 3 mice hyaline corpora lutea were found. An artery in a hyaline-gelatinous corpus luteum was surrounded by hyaline material. In 2 animals, 5 and 6 months of age, hyaline corpora lutea were not seen, but hyaline material surrounded some arteries.

Period 2. In a large majority of the mice hyaline corpora lutea, showing various stages of hyalinization or gelatinization, were present. But also young, well preserved corpora lutea were found together with hyaline corpora lutea in a number of animals. The young corpora lutea were especially frequent in the younger mice of this period. In the hyaline corpora lutea connective tissue could disappear in the periphery, while the more central gelatinized part could be more or less organized and replaced by connective tissue, or in other instances the gelatinous areas could soften and liquefy still further. Connective tissue grew also into the margin of the preserved corpora lutea. In the periphery of one corpus luteum there was a zone containing clumps of blood, which were surrounded by foreign body giant cells. Hyalin appeared also around a vessel. Besides the hyaline degeneration of corpora lutea, other types of regression were seen in some of the corpora lutea.

Period 3. (a) Mice aged 17 to 19 months: In the majority of ovaries hyaline-gelatinous material predominated; in such areas the connective tissue could either still be present or be dissolved. But in some ovaries a number of red-staining mature corpora lutea or some of their remnants could still be seen. In other ovaries even partly preserved corpora lutea were absent. In 1 mouse there were structures which may have been luteinized follicles (pseudo corpora lutea). In one ovary small, not yet gelatinized corpora lutea were preserved, but very cellular connective tissue strands invaded these structures and destroyed them to a considerable extent. Also vacuolar degenerated corpora lutea were seen. Some pinkish-staining areas consisting of vacuolar cells, which contained yellow blood pigment, probably represented theca interna cells of atretic follicles. In hyaline-gelatinous material, present in the ovary and composed of conglomerated, degenerated corpora lutea, a completely necrotic artery containing some bluish calcium deposits in its walls was seen. In other gelatinous corpora lutea a few preserved capillaries could still be present. (b) Mice aged 15 to 17 months: Here again, much hyalinization and gelatinization were found in the ovaries, but a number of good corpora lutea and even of rather young corpora lutea occurred; in such a structure a mitotic figure was seen in a corpus luteum cell. There were also large areas of vacuolated cells, which represented remnants of degenerated vacuolar corpora lutea. In places it seemed that also interstitial gland was in part hyalinized. In 3 mice belonging to this age group no corpora lutea were found.

Period 4. In 3 nonbreeding mice either no corpus luteum was present or there were remnants of corpora lutea, and in one ovary there was probably a luteinized follicle representing a pseudo lutein body. In the remaining 5 mice there were corpora lutea in various stages of gelatinization, but there were also some vacuolated corpora lutea or even some areas of well preserved corpus luteum tissue. In a breeding mouse there was a largely gelatinized remnant of corpus luteum tissue; besides, the greater part of the ovary was hyalinized or gelatinized, with hyalinization affecting also the stroma. In the gelatinized material cavity formation could take place.

COMMENT

Some of the conclusions which may be drawn from the changes observed in ovaries of the various strains will now be set forth under appropriate headings.

Development of Follicles in the Different Periods of Life.—In periods 1 and 2, as a rule, the follicles of the ovaries of the large majority of mice of all the strains develop in a normal manner and some of these reach full size. However, in very young mice, those below the age of 2 months, or in mice which have just ovulated, or even in somewhat older mice which are underweight, and occasionally also in other mice, fully developed follicles may be lacking. In strain D in a number of breeding mice large follicles were not seen in period 4, and besides there was a reduction in the number of follicles in some of the animals. There was no noticeable difference between high and low rate mammary cancer strains as far as the development of follicles is concerned in the first year of life. In period 3 some decline takes place in the development of the follicles, but even in this period follicles may still be well developed, as, for instance, in strains C and AKA. In strain C57 a considerable number of mice had large follicles, but in others they did not reach full size or the number of follicles was reduced. In strain C3H all mice had large follicles, while in strain D large follicles were lacking in the large majority of mice and in 2 mice follicles were lacking altogether; also in strain Old Buffalo large follicles, as a rule, were lacking. Thus in period 3 there is a definite decrease in the number of mice in which large follicles have developed but there is a considerable difference in the findings in different strains without regard to whether they are high or low mammary cancer strains.

In period 4 the decline in the development of the follicles is more pronounced than in period 3. Thus during period 4 in strain AKA follicular atresia set in usually prematurely. In strain Old Buffalo no follicles or only very small ones were seen in the oldest mice of this period, although in the younger animals large follicles could develop. In strain CBA no follicles were seen in the ovaries of the oldest mice, but even in the younger animals follicles did not develop in a normal manner. In strain C57 the result was better in period 4. In about one half of the mice large follicles developed in the first half of this period, whereas in the second half only small or medium-sized follicles were seen. In strain D no follicles were found except in 1 mouse, in which undeveloped follicles were present.

Presence of Mature or Recently Ruptured Follicles.—In periods 1 and 2 mature or just ruptured follicles could be seen in nonbreeding as well as in breeding mice. In animals 5 weeks of age or younger ovulation usually did not seem to occur. In period 3 mature or just ruptured follicles appeared only in strains C57, D and A. In strain A a mature follicle was noted in 1 mouse. There was then a sharp decline in the occurrence of mature follicles in the transition from period

2 to period 3, and a still sharper decline in passing from period 3 to period 4; but there was no great difference in these respects between high and low mammary carcinoma strains.

Presence of Larger Than Normal or of Cystic and/or Hemorrhagic Follicles and the Phagocytic Activity of Granulosa Cells.—Hemorrhages in follicles are often associated with enlargement of follicles which is probably the primary change in such cases, although it is possible that in some instances a primary hemorrhage may have caused distention of a follicle. Hemorrhages are seen also in atretic follicles, where they may surround the egg. It is probable that in atretic follicles hemorrhages usually result when capillaries grow through the enlarged and perhaps vacuolated theca interna into the membrana granulosa, in the end reaching the central cavity. There may be a primary hyperemia which may be followed by hemorrhage. It is probable that in large follicles hemorrhages may be caused by hyperemia, such as occurs preceding ovulation. The enlargement of follicles and especially their cystic character are presumably due to a deficiency in the processes of maturation and ovulation, and such a deficiency may be caused by deficient function of the anterior lobe of the pituitary gland. Enlarged and cystic follicles often occur unaccompanied by hemorrhage, and, on the other hand, hemorrhage may occur in follicles of normal size. In 1 animal the clotted blood present in the cavities of several follicles had assumed a lamellated structure, and these blood clots were surrounded by phagocytic cells carrying blood pigment. In some instances it appeared that granulosa cells in follicles undergoing atresia were able to take up red blood corpuscles and also disintegrated cell material. Cells which were still part of the intact membrana granulosa were active rather than detached granulosa cells, although in the guinea pig the latter may also take up disintegrated cells during atresia of the follicles. These abnormal types of follicles may occur at all periods in the life of the mouse, but there are some differences as to the frequency with which they appear at various periods. All these types of follicles, but in particular very large follicles and occasionally even hemorrhagic follicles, may be found as early as period 1, but on the whole they are rare at this time of life. The majority appear in period 2 and still more in periods 3 and 4. Especially the cystic and cystic-hemorrhagic follicles are not usually seen in young mice. Likewise hemorrhages in atretic follicles are as a rule noted only from period 2 on, although they may appear even in period 1.

Occurrence of Primordial Follicles in the Ovaries at Various Periods of Life.—Primordial follicles or follicles approaching this state are not limited to period 1 but occur also in all the later periods. In period 4 they were seen in several strains, especially in the deeper parts of the

ovary between columns of interstitial gland cells and near the medulla. It is of interest to recall here the fact that I occasionally observed in the lumens of vessels, and likewise in the deeper portions, of the ovary of the guinea pig structures which apparently represented primordial follicles.

Atresia of Ovarian Follicles.—I have referred already to a characteristic difference of the follicular atresia in the mouse and the guinea pig. In the latter a massive disintegration of the granulosa cells takes place shortly after the onset of atresia, which is followed by phagocytosis of the disintegrated cells. In the mouse, on the other hand, the membrana granulosa may remain preserved for a relatively long time, but in the end connective tissue and blood capillaries may grow through the preserved granulosa and fill the cavity of the follicle. However, even in the mouse the onset of atresia does not as a rule leave the granulosa cells quite unchanged. They may undergo vacuolation with or without yellow pigment being deposited in the vacuolated cells. These atretic changes may occur during all periods of life. There may also take place, even during early stages of atresia, a solution of vacuolated granulosa cells, and this degeneration may occur in early as well as in later periods of life. In 1 case granulosa cells which were filled with small granules became transformed into colloid-like material. In hemorrhagic atretic follicles blood pigment may be the source of the yellow pigment seen in the granulosa. In strain C57 I observed that adjoining granulosa cells could unite into a syncytium and that the nuclei then could assume a rosette-like arrangement. It is significant that in strain C57 cells of the adrenal cortex situated near the medulla could join to form similar syncytia and rosettes. However, in a single instance the same change was observed in a follicle in strain C3H. As stated, hemorrhages were seen likewise in atretic follicles, and the hemorrhagic changes occurred especially in periods 3 and 4, therefore in older animals. But sometimes they occurred in period 2 as well. The hemorrhage under these conditions probably occurred when capillaries were growing into the granulosa and the coagulated blood could directly surround the egg. Under such conditions the vacuolar granulosa cells may take up red blood corpuscles or blood pigment. In rare cases granulosa cells were observed moving between the segments of segmented eggs in the centers of atretic follicles, and in 1 case a centrally situated egg was being destroyed by connective tissue growing through the granulosa. Connective tissue may begin to extend into the granulosa during early stages of atresia; gradually it reaches the inner aspect of this tissue and there forms a connective tissue membrane. If the inner layers of the granulosa have been destroyed, connective tissue may fill the cavity which has thus developed. With the connective tissue capillaries may penetrate into the granulosa and

hemorrhage may occur not only in the granulosa but in the theca interna of atretic follicles. Furthermore,* during atresia the cells of the theca interna may enlarge and become vacuolar, and they may even carry yellow pigment, changes similar to those seen in the granulosa. In some instances it is possible to distinguish, in atretic follicles, theca cells from granulosa cells by the large size of the latter; moreover, as a rule, blood vessels are found in the theca. These changes in the theca cells may be noticeable as early as in period 1, but usually they develop only during periods 2, 3 and 4. The enlarged cuboidal theca cells can be seen at different stages to give origin to interstitial gland.

In some cases the enlargement of the granulosa cells and theca cells, together with the capillaries growing into the granulosa, and the lutein-like appearance of the latter, may lead to the formation of pseudo corpora lutea. This process differs from true ovulation in that rupture of the follicle and discharge of the egg do not take place. In some instances, however, it may be difficult to distinguish between an atretic follicle and a pseudo corpus luteum. In rare cases an atretic follicle may be transformed into a hyaline structure, and in 1 instance a hyaline layer was observed separating membrana granulosa and theca interna in an atretic follicle. Quite rarely nests of degenerated yellow granulosa cells can be recognized within the strands of interstitial gland tissue. In 2 mice which had been given injections of solutions of estrone and extracts of the anterior lobe of the pituitary gland for a considerable number of months the development of follicles ceased when they had reached small to medium size.

Life Cycle of the Corpora Lutea.—The corpora lutea result from the rupture of mature follicles. They grow, mature and then degenerate and gradually disappear. If stained with hemotoxylin and eosin during the process of their growth, they tend to take on a bluish tint; with increasing maturity this gives way to the red tint of eosin. At first the blood circulates well through corpora lutea; the capillaries are wide; at a later stage, however, when the decline of these bodies sets in, the capillaries become narrow and may contain polymorphonuclear leukocytes in large numbers. In this description of the life cycle of corpora lutea mention of their peculiarities during pregnancy has been omitted. In some cases it may be difficult to distinguish true corpora lutea from the pseudo corpora lutea which originate in atretic follicles through the enlargement of the granulosa cells, which, as stated, in certain instances become lutein-like; at the same time the theca cells may enlarge and become vacuolated. An egg is present in the center of such a structure. However, at least in the guinea pig it can be seen that in true corpora lutea, which have developed from follicles as a result of ovulation, the egg may occasionally be retained in the center of

a ruptured follicle. Thus in the mouse the presence or the absence of an egg may not furnish a decisive distinction between true and pseudo corpora lutea.

In some cases, furthermore, it is difficult to distinguish between a true corpus luteum and lutein-like interstitial gland tissue. This difficulty may arise in several conditions: (1) if the similarity between interstitial gland tissue and corpus luteum tissue is so great that only the sharp localization of the corpus luteum makes it possible to differentiate between these two formations, and this may not always be an easy task; (2) if the corpora lutea are pushed from the periphery of the ovary toward the center and here begin to degenerate, or if remnants of degenerated corpora lutea are included in the interstitial gland as one of its constituents; (3) if, during the degeneration of the corpora lutea, the latter are invaded and become partly replaced by interstitial gland; (4) if, as occurs under certain conditions, not only the true corpora lutea but also certain areas in the interstitial gland undergo a peculiar type of degeneration leading to hyalinization and gelatinization.

Mode of Degeneration of the Corpora Lutea.—Under ordinary conditions three processes take place during the degeneration of the corpora lutea: (1) vacuolation of the cells of the corpus luteum, which may begin in the central part of the corpus; (2) connective tissue invasion of the degenerating corpus luteum, followed (3) by shrinking of this structure. These processes constitute the usual degenerative change. At the same time the degenerating corpora lutea may be pushed from the peripheral parts of the ovary, the cortex, toward the center and the medulla, where they ultimately disappear; as stated, not only the connective tissue may invade perishing corpora lutea, but also the surrounding interstitial gland may do so, and in the end interstitial gland tissue takes the place of the disappearing corpus luteum tissue.

Hyalinization and Gelatinization of the Corpora Lutea.—There occurs in addition to the usual process just described a second type of degeneration consisting in hyalinization and gelatinization of these structures, leading at least in some cases to solution processes and occasionally also to deposition of calcium salts in the degenerated areas. This hyalinization can begin with deposition of a hyaline material in or around the walls of the arteries and of the capillaries within the corpora lutea; hyalin or a hyalin-like substance seems to develop also within the lutein cells. This mode of degeneration starts apparently in the peripheral parts of the corpus luteum and progresses toward the center. The stage of hyalinization is followed by a taking up of fluid by the hyaline tissue elements, leading to gelatinization, which may affect also the stroma of the corpora lutea. If this progresses, complete liquefaction of the corpus luteum and loss of nuclei, which prevent

distinguishing between corpus luteum tissue proper and stroma, take place. A precipitation of calcium salts may occasionally occur not only in the vessel wall, after parts of it have become necrotic, but also in the corpus luteum proper. In the end connective tissue may invade the hyaline-gelatinous corpora lutea from the periphery.

Various Other Hyaline Changes in the Ovary.—Often hyaline changes occur simultaneously in a number of corpora lutea of an ovary, so that a large portion of this organ as a whole becomes converted into hyaline or gelatinous material; finally the stroma elements which at first separate the various corpora lutea may disappear. Instead of steadily progressing general hyalinization-gelatinization, there were exceptionally noted some clumps of hyaline material in an otherwise not yet hyalinized corpus luteum, and foreign body giant cells could surround these hyaline clumps. But, in addition, more diffuse hyaline areas may occasionally be seen in ovaries, apparently unconnected with hyaline corpora lutea. Such hyaline areas may be present in various parts of the ovary, even at a distance from hyaline corpora lutea; they are especially prone to appear in older mice, and here hyaline blood vessels may extend into the tissue surrounding the ovary.

These arterial changes evidently correspond to the hyalinization of vessels which may appear in older individuals of other species. The question now arises (1) whether the hyalinization of an artery is the same process as the hyalinization of the corpora lutea, and (2) whether this process is a specific one of hyalinization or whether it represents an amyloid change. In the adrenal gland of the mouse my associates and I have attempted to distinguish between amyloid and hyaline changes by means of specific amyloid staining reactions, but without success. Also some other investigators did not succeed in obtaining the typical amyloid-staining reaction in the mouse. Under these conditions the question as to the chemical nature of this hyaline substance must be left undecided; but it is probable that the hyaline changes observed in the arteries of the corpora lutea and in other structures of the ovary and surrounding tissue are closely related to the hyaline changes which are found quite generally in blood vessels with advancing age. Processes of hyalinization and gelatinization have not been observed in the corpora lutea of the guinea pig.

Mode of Degeneration of Corpora Lutea in Various Strains of Mice.—When the various strains of mice were compared as to the mode of degeneration of the corpora lutea, no strain was found to be entirely free of the usual mode of degeneration, consisting in vacuolation, connective tissue ingrowth and shrinkage. Conversely, there was no strain in which hyaline or gelatinous changes failed to occur in corpora lutea, except perhaps strain CBA; but even in this strain it is not certain

that hyalinization might not have been observed if a still larger number of animals had been examined, especially in the later periods of life. On the other hand, the various strains differed greatly in the frequency with which these changes occurred in the corpora lutea and in the degree to which this mode of degeneration predominated over the usual one. Relative to the frequency and intensity with which hyalinization appeared, the various strains can be arranged approximately in the following order: CBA < C < C57 black < Old Buffalo < New Buffalo < C3H and AKA < D < A. In some cases the difference between adjoining strains was not definite. There is a strong indication that on the whole strains possessing a low incidence of mammary carcinoma have less tendency toward hyalinization than do the high mammary tumor strains. Thus the mammary tumor rate is low in strains CBA, C, C57 and Old Buffalo. In strain New Buffalo there is a medium mammary tumor rate. In strains C3H, D and A breeding mice the mammary tumor rate is high. However, while this relation between tumor rate and hyalinization is indicated, it is not absolute. Thus strain AKA, which possesses a very low mammary tumor rate, exhibits a strong tendency toward hyalinization and gelatinization of corpora lutea. Among the other strains there is no complete correspondence between mammary tumor rate and their tendency toward hyalinization and gelatinization. Strain CBA has less tendency toward hyalinization of corpora lutea than have strains C57 black and C, although the two last-named strains have a lower tumor rate than strain CBA. The results of this comparison again show that the use of only a small number of strains may lead to erroneous conclusions as to the correlation between certain strain characteristics.

Relation of Age to Incidence of Hyalinization.—In general the frequency of hyalinization of corpora lutea increases with age of the mice and this relation applies to strains in which hyalinization is a common occurrence as well as to those in which it is found relatively infrequently. However in those strains in which relatively few animals are affected, the occurrence of chance conditions may cover up the importance of age. Thus in strain C hyalinization occurred in period 1, although in this strain the change is on the whole rare. In period 3 an apparent transition of a normal to a gelatinous organ was noted, but there was no complete hyalinization in this case. As stated, in strain CBA no hyalinization was observed. In strain C57 there was in period 3 some hyalinization of corpora lutea as well as of arteries. Furthermore, in some other mice hyaline areas extended from the hilus upward toward the periphery. In period 4 no corpora lutea were recorded. In strain Old Buffalo 1 mouse was seen with hyaline corpora lutea in period 2. In period 3 of this strain there were some strands of hyaline tissue. In

period 4 a large gelatinous corpus luteum and a remnant of a gelatinous corpus luteum were noted. In strain New Buffalo about one third of the corpora lutea were hyaline or gelatinous in period 2. In period 4 large clumps of hyaline tissue were seen. In strain C3H some mice exhibited the usual mode of regression, while others had hyaline and gelatinous corpora lutea, in period 2. The greater part of the corpora lutea could be gelatinous; hyaline deposits occurred around vessels, and necrotic areas were found in hyaline vessel walls. In period 3 most corpora lutea were hyaline or gelatinous. In period 4 only 1 mouse was left for examination, and hyaline-gelatinous corpora lutea were seen in this animal. In strain AKA, in period 1 the usual mode of degeneration of corpora lutea predominated, but hyalinization did take place in some animals; also, transitional stages were noted. In period 2 the majority of the mice had corpora lutea in various stages of hyalinization, and in some animals almost all the corpora lutea were hyalinized. Also, there were hyaline arteries in association with hyaline corpora lutea. In period 3 in a large majority of the mice the corpora lutea were hyalinized, but some vacuolation still took place. Hyaline arteries were found in the ovarian tissue outside of corpora lutea. In period 4 there were shrunken remnants of corpora lutea. In gelatinous corpora lutea there were necrotic areas surrounded by foreign body giant cells. In strain D, in period 1 neither hyalinization nor gelatinization occurred. In period 2 the corpora lutea were mostly hyaline or gelatinous, but in rare instances degeneration by vacuolation took the place of hyalinization. In period 3 there were mostly hyaline or gelatinous corpora lutea, but there were also shrunken vacuolar corpora lutea with connective tissue ingrowth. In period 4 hyaline-gelatinous corpora lutea were found besides remnants of such corpora lutea. There was also hyalinization of vessel walls. Solution processes took place, and in other areas precipitation of calcium salts was present in gelatinous corpora lutea. In strain A, in period 1 degeneration of corpora lutea occurred mostly by vacuolation and connective tissue ingrowth, but in several older mice of this period hyaline or gelatinous corpora lutea were noted. In 1 mouse in which no hyaline corpora lutea were observed, a hyaline artery was seen, but hyalinization of arteries occurred also in hyaline corpora lutea. In period 2 a large majority of the corpora lutea were hyalinized or gelatinized, but in some ovaries vacuolar corpora lutea also were found, in addition to preserved corpora lutea. In an older mouse necrosis and calcium deposits were seen in the walls of an artery. There was also some vacuolation of corpora lutea. In period 4, likewise, a large majority of corpora lutea were hyaline or gelatinized; there were also isolated clumps of hyaline tissue, but even at this late time of life there were a few well preserved corpora lutea besides some remnants of vacuolar corpora lutea.

To summarize: In period 1 no hyalinization of corpora lutea was found in strains CBA, C57, Old Buffalo, New Buffalo, C3H and D. In strain C an exceptional case of hyalinization was observed. In strain AKA the usual mode of degeneration predominated, but hyalinization of corpora lutea occurred in some cases; also, transitional stages were seen. In strain A degeneration of the corpora lutea, as a rule, took place by vacuolation and connective tissue ingrowth, but in several older mice of this period hyaline or gelatinous corpora lutea were found. During period 1 there is, then, as a rule, absence of hyaline corpora lutea; only in strains AKA and A are mice affected by hyalinization or gelatinization of these structures, and those affected are but a small minority. Hyalinization and gelatinization of the corpora lutea therefore depend not only on the age of the corpora lutea but also on the age of the animals. In this sense hyalinization and gelatinization of corpora lutea may be considered as a phenomenon of aging.

In periods 2 and 3 the number of corpora lutea present was relatively great in the various strains and the animals had reached an older age. Accordingly the absolute number of hyalinized corpora lutea reached a maximum at this time. This was especially evident in strains in which the tendency toward hyalinization was strong, but even in the other strains the proportion of hyalinized and gelatinized corpora lutea was large. In strain C no typical hyalinization was found, but a corpus luteum showed a condition transitional to hyalinization in period 3. In strain CBA gelatinization was not observed. In strain C57 some hyalinization was noted in the corpus luteum in period 3. In strain Old Buffalo a single hyaline corpus luteum occurred in period 2. In period 3 some strands of hyaline tissue were seen. In strain New Buffalo about one third of the corpora lutea were hyalinized or gelatinized in period 2, while in period 3 hyaline-gelatinous corpora lutea were found in 2 mice. In strain C3H a minority of animals had hyaline or gelatinized corpora lutea in period 2, but in some of these animals the greater part of the ovary was gelatinized. In period 3 the majority of the corpora lutea were hyalinized or gelatinized and in some cases even the greater parts of the ovaries were gelatinized. In strain AKA, in the majority of the mice the corpora lutea were in various stages of hyalinization, and in some animals almost all the corpora lutea were hyalinized in period 2. In period 3, likewise, the majority of the corpora lutea were hyaline or gelatinous. In strain D much hyalinization and gelatinization of corpora lutea was seen during periods 2 and 3, and in strain A the large majority of the corpora lutea were hyalinized or gelatinized during periods 2 and 3. In strain New Buffalo there was a somewhat greater intensity of hyalinization than in strain Old Buffalo, and in strain AKA the hyalinization seemed to be more intense than that in strain C3H. Hyalinization was pronounced in strains D and A.

In period 4 degeneration of corpora lutea, presence of remnants of corpora lutea and in the end their complete destruction were noticeable. Well preserved corpora lutea were not frequent at this period. In strain CBA no corpora lutea were found in a number of mice; in others there was connective tissue ingrowth in degenerated corpora lutea, and remnants of corpora lutea were present; hyalinization was lacking. Only in 1 mouse were corpora lutea staining red with eosin seen. In strain C57 there were neither preserved corpora lutea nor the usual kind of vacuolated or hyaline-gelatinous corpora lutea, but in 1 animal two young vacuolar corpora lutea with some central hemorrhage were seen. In strain Old Buffalo there were large gelatinous corpora lutea and remnants of such corpora lutea, also vacuolar corpora lutea. In some mice leukemia caused lymphocytic invasion and destruction of corpora lutea. But there were 4 mice (2 were 28 months and 2 were 20 and 21 months of age) in which preserved corpora lutea were found. In some instances the distinction between true corpora lutea and corpus luteum-like interstitial gland tissue was difficult. In strain New Buffalo there were mostly degenerated vacuolar or hyaline remnants of corpora lutea, in addition to a corpus luteum that was invaded and split up by connective tissue. In strain C3H, in the single mouse left in this period, some good, as well as some hyaline-gelatinous, corpora lutea were found. In strain AKA no preserved corpora lutea were noted, but there were remnants of vacuolated and shrunken corpora lutea. There were also hyaline-gelatinous corpora lutea as well as remnants of such corpora lutea. In 1 breeding mouse a great part of the ovary consisted of gelatinous corpora lutea in which the nuclei had been lost. These necrotic areas were surrounded by foreign body giant cells. In strain D there were some preserved small corpora lutea, but the larger corpora lutea were hyaline or gelatinous and in some places calcium deposits were found in gelatinous corpora lutea; also, solution processes, accompanied by loss of nuclei and followed by ingrowth of connective tissue, could occur in gelatinous corpora lutea. In 2 mice the corpus luteum tissue was tumor-like; it contained large nuclei and seemed to be slowly growing. In strain A the large majority of corpora lutea were hyaline and gelatinous. This process could advance so far that the greater part of the ovary became gelatinized and partly dissolved. A few small preserved corpora lutea were seen, however, in addition to some remnants of vacuolar corpora lutea. There were observed also clumps of hyaline material, which represented probably remnants of hyaline corpora lutea.

In these strains no strict parallelism between the tendency toward hyalinization of corpora lutea, the formation of new corpora lutea and the presence of preserved corpora lutea was seen. Thus in strain AKA, in which hyalinization was pronounced, preserved corpora lutea were

lacking in period 4. Furthermore, while there were some indications that the chances are greater that corpora lutea may be preserved at a later age of the mice in strains showing a high incidence of mammary carcinoma than in low mammary carcinoma strains, such a distinction between these two classes of strains is not definite. While in the high mammary cancer strains A, D and C3H preserved corpora lutea were found in period 4, some preserved corpora lutea occurred also in the low mammary cancer strains CBA and Old Buffalo during this period. Moreover in none of the strains studied during this time of life were there seen any new corpora lutea in contrast to the findings in period 3, where new corpora lutea appeared not infrequently in various strains, irrespective of whether these strains had a high or a low mammary cancer incidence or whether hyalinization of corpora lutea was a common or a more rare occurrence. In general in period 4 there was predominance of the various degenerative processes.

Growth Processes in Corpora Lutea.—In the follicles and corpora lutea abnormal, tumor-like growth processes were absent as a rule. However, as stated already, in strain D there were found in 2 mice in period 4 nuclear hypertrophy and increased size of corpora lutea. In former investigations occasional mitoses were noted in corpus luteum cells of the guinea pig. There have now been found mitotic figures in corpora lutea in period 1 in three strains of mice; in strain D they were observed in well preserved adult corpora lutea, and in strain A a mitotic figure was seen in a young corpus luteum.

SUMMARY

A decline of the full development of follicles sets in in period 3 and becomes more pronounced in period 4. However, differences are observed in this respect between different strains; there is no parallelism between the fate of the follicles in different strains and the tendency of these strains to have mammary carcinoma. A sharp decline in the number of mature follicles takes place during period 3 and still more during period 4. While in this respect, also, a difference is observed in different strains of mice, it does not correspond to the division of mice into high and low mammary carcinoma strains.

Follicles may be very large, and cystic or hemorrhagic, or cystic and hemorrhagic at the same time. It seems that in some cases dilatation of the follicles is the primary process, while in other cases hemorrhage is primary. Hemorrhages may also be found in atretic follicles. Under certain conditions the granulosa cells may act as phagocytes toward constituents of the extravasated blood or they may take up disintegrated cell material.

In mice primordial follicles may be seen in the deeper parts of the ovary, even in older animals; likewise in the ovary of the guinea pig

they have been observed in the blood vessels in the deeper parts of the ovary.

The atresia of follicles of mice differs in some respects from the corresponding process in guinea pigs. A number of changes are described which may be observed in atretic follicles in mice.

The corpora lutea of mice undergo a definite life cycle, but in some cases it may be difficult to distinguish between corpora lutea and pseudo corpora lutea or between true corpora lutea and certain types of interstitial gland.

Besides the usual type of degeneration of the corpora lutea, in which essentially three processes take part, there is a second type consisting in processes of hyalinization, gelatinization and solution. The hyalinization of the corpora lutea may begin in or around their blood vessels, and the problem arises as to the nature of the hyalinization occurring in the blood vessels and in the corpora lutea proper; in the latter hyalinization is perhaps related to amyloid changes, which in the mouse cannot—at least not in all cases—be clearly distinguished from certain other processes by specific staining reactions.

Both types of degeneration of corpora lutea were encountered in all the strains examined except perhaps strain CBA, in which so far neither hyalinization nor gelatinization has been observed. However, the proportions in which these two types, represented by vacuolation and hyalinization, occur differ greatly in different strains. On the whole hyalinization and gelatinization predominate in strains possessing a high mammary tumor rate; but this difference between high and low mammary tumor strains is not absolute. Strains AKA, which has a very low mammary tumor rate, has a strong tendency toward hyalinization of the corpora lutea, and strain CBA, which has a higher tumor rate than strain C57 black, has less tendency to hyalinization of the corpora lutea than strain C57. A consideration of only a small number of strains would therefore lead to erroneous conclusions as to the correlation between the frequency of occurrence of certain characteristics and some other properties of these strains.

The hyalinization and gelatinization of corpora lutea increase in frequency and intensity with increasing age of the mice up to period 4. In the last period of life destructive processes predominate in corpora lutea. There was no complete correspondence between the greater or the lesser tendency of corpora lutea to undergo hyalinization and the presence of preserved corpora lutea in the various strains of mice.

Tumor-like formations of corpora lutea were found in only 2 mice of strain D.

Mitotic figures can be seen in corpora lutea of mice as well as in those of guinea pigs, although they are rare in both species.

II. THE FATE OF THE INTERSTITIAL GLAND AND THE FORMATION OF OVARIAN DUCTS AND CYSTS

IN THE first part of these investigations the fate of follicles and corpora lutea was described, and it was shown that with advancing life of the mice follicles and corpora lutea disappear more and more and their place is taken by the interstitial gland and various duct formations. In this part, the changes taking place in the latter two substitutional structures will be followed from the first to the fourth period of life. I shall proceed by describing more fully the alterations which occur in these two formations in one low mammary cancer strain, CBA, and in one high mammary cancer strain, D, and I shall then compare the development of the interstitial gland and the ducts in these two strains with their evolutions in the other strains.

OBSERVATIONS IN STRAINS CBA AND D

STRAIN CBA.—*Period 1.*—As usual, during period 1 the interstitial gland consisted of small and medium-sized, more or less cuboidal cells. Groups of typical yellow-pigmented interstitial gland cells were mostly lacking, but some yellow degenerated granulosa cells were seen in atretic follicles, which were found occasionally among the groups of interstitial cells.

Ducts derived either from germinal epithelium or from medullary tissue were not conspicuous.

Period 2.—The interstitial gland was similar to that seen in period 1, but in 5 of 14 mice yellow cell nests developed in the interstitial gland. In 1 mouse there were merely some yellowish brown cells, which seemed to be remnants of the membrana granulosa of atretic follicles; also in another mouse granulosa of atretic follicles seemed to be present in the interstitial gland. In still another animal there were in various places of the interstitial gland packages of yellow and pinkish vacuolar cells, which may have been derived from old corpora lutea. The yellow cells seen in this strain were smaller and less numerous than those in strain C57 black, and the vacuolar yellow cells were larger than the ordinary interstitial gland cells. These yellow cells, also, may have been derived at least in part from the granulosa of atretic follicles.

In 3 mice medullary cysts were found; but in only 1 animal were ducts seen in the cortex.

Period 3.—In the older group of mice, 17 to 20 months old, the ovaries consisted mostly of interstitial gland tissue. This was in part composed of small cuboidal cells with vesicular nuclei. These cells became larger in the cortex, and there were here more groups of yellow vacuolar cells admixed to these cells, especially near the subgerminal spindle cell tissue. Some of these yellow cell nests may have been derived from atretic follicles. In general there were large amounts of yellow interstitial gland tissue in these ovaries. Also in the younger mice, 15 to 17 months of age, the interstitial gland tissue represented the largest part of the ovary. The amount of yellow vacuolar cells was perhaps somewhat smaller than

in the older mice, but there was great variation in this respect. In the cortex there was a small number of groups of reddish and small, pale yellow cells; they may perhaps have been remnants of corpus luteum tissue. But most of the interstitial gland consisted of small or medium-sized cells or of somewhat larger cells: the last appeared almost lutein-like. Different degrees of vacuolation were found, and cellular fibrillar connective tissue strands separated various areas in the lutein-like tissue. In the center of the ovary dense hyaline material was found; it may have been derived from atretic follicles. In some places there were groups of cells which resembled large granulosa cells in transition to corpus luteum tissue.

Several medullary ducts were found. Cortical ducts accompanied by subgerminal spindle cell tissue could be seen to move downward into the deeper ovarian areas; these ducts could be lined with cuboidal epithelium. Medullary ducts surrounded by interstitial gland tissue extended upward toward or into the cortex. These ducts were lined with cylindric epithelium or with yellow vacuolar cells; the higher up they penetrated the smaller they became. The layer of subgerminal spindle cells sent processes downward; they surrounded cell nests, possibly remnants of some atretic follicles.

Period 4.—Interstitial gland structures were here so intimately mixed with duct formations that these two constituents could not always be sharply separated. In the older group of mice, ranging in age between 26 and 31 months, there were adenomatous formations situated in groups of interstitial gland cells and covered by germinal epithelium; these formations protruded into a germinal cyst overlying the ovary. It seemed that some ducts, lined with cuboidal vacuolar cells and surrounded by subgerminal spindle cell tissue, gave origin to these adenomatous structures. Papillae filled with yellow interstitial gland structures protruded in places into these germinal ducts. Some ducts were lined with flat epithelium, and in others there were pearls consisting of squamous epithelium, which could be partly hyaline. Mitotic figures were seen in the epithelial lining of the ducts. In some places the duct epithelium became vacuolated and yellow. It seemed that groups of yellow cells situated in the ovarian stroma were derived from such ducts filled with vacuolar yellow cells. Some of these vacuolar cells could coalesce and form syncytia. In other places interstitial gland consisted of small cuboidal cells possessing small nuclei; these cells were probably derived from the theca interna of atretic follicles. Groups of somewhat larger cells may possibly have represented remnants of corpus luteum tissue. The groups of yellow cells were in places so large that they exerted pressure on the connective tissue stroma and destroyed it. But also medullary ducts were in intimate connection with interstitial gland, and it was difficult to determine whether they were merely in close contact with it or whether they changed into this tissue and thus gave origin to it. Underneath the germinal epithelium there could be found either the usual layer of spindle cells or a layer of hyaline material. The spindle cell tissue often penetrated deep into the ovary, either around ducts or between strands of interstitial gland, forming partitions between the latter. Deeper toward the center of the ovary connective tissue fibers gradually developed between the spindle cells. Likewise hyaline strands penetrated into the interstitial gland and separated it into bundles of interstitial gland cells. In the younger group of animals conditions on the whole were similar to those observed in the older group, but in the younger animals the structure of the ovary was perhaps a simpler one,

inasmuch as groups of smaller or medium-sized cuboidal cells became more prominent. These formations resembled those seen in earlier age periods. However, in these animals also vacuolation and formation of yellow pigment occurred. It seemed that the yellow cells could in some instances contain blood pigment. Likewise medullary ducts lined with vacuolar cells seemed to give origin to interstitial gland.

As to ducts and cysts, in the older mice one occasionally found germininal epithelial cysts and also medullary cysts. Yellow vacuolar cells lined some of the ducts, and large vacuolar cells were seen in the lumens of the tubules. There were also coiled ducts containing yellow cells, and mitotic figures were seen in ducts. Pearl-like bodies which centrally contained keratin or concentric lamellar hyaline material were found in large, cystically dilated ducts and were derived from their lining cells. Strands of subgermininal spindle cells could accompany the ducts, and some of the latter penetrated into the fat tissue surrounding the ovary. Ducts lined with high cylindric epithelium or, in other cases, with vacuolar light-colored or yellow cells originated probably from medullary ducts. They penetrated upward into the interstitial gland and seemed to destroy it in places. Mitotic figures occurred in the epithelium of the medullary ducts. A great part of the ovary consisted of small ducts lined with low cuboidal epithelium; they were surrounded by subgermininal spindle cell tissue. These ducts were probably derived from germininal epithelium. They grew downward into the center of the ovary, while medullary ducts could penetrate upward, even as high as into the subgermininal connective tissue. Some of the germininal ducts could be coiled, and they could be surrounded by dense hyaline strands which took the place of the spindle cells. Both medullary and germininal ducts seemed to change in various places into interstitial gland cell tissue, and thus they were apparently able to produce interstitial gland tissue, including yellow cell strands. Medullary ducts seemed to form also lutein-like tissue or to penetrate into packages of yellow cells.

STRAIN D.—Period 1.—The interstitial gland cells were mostly small or medium sized, although their size varied to some extent in different places; in some areas they were granular or filled with small vacuoles. In mice 3 or 4 weeks old the interstitial gland was as yet rudimentary or lacking altogether. Yellow interstitial gland cells were in evidence only in 1 nonbreeding mouse, not quite 8 months of age and weighing only 17 Gm., and in 2 breeding mice. There seemed to exist an inverse relation between the size of the follicles and the amount of interstitial gland that had developed. In 2 animals the vessels of the medulla were much enlarged.

Ductlike processes given off by the germininal epithelium and penetrating deeper into the ovarian tissue were not recorded; but in 1 mouse a large medullary cyst was observed.

Period 2.—The interstitial gland resembled that seen during period 1. In a number of mice there were groups of larger, usually vacuolated yellow cells, the pigment being derived in some instances from blood pigment taken up by these cells, which were thus acting as phagocytes. Such yellow cells were found in the medulla, especially around the vessels. But also some regressing corpora lutea seemed to have been incorporated in the interstitial gland tissue.

Ducts, in contrast to the first period, in which they were absent or rare, were more frequent in period 2. Among the older mice they were prominent in 5 animals. In one of these there were in the cortex convoluted ducts, which were probably derived from the germininal epithelium. In a second one there were, in addition to invaginations of the germininal epithelium, medullary ducts with papillae and a large papillary cyst of medullary origin. In a third mouse there was a duct, probably of medullary origin, lined with cells resembling sebaceous

gland cells. Likewise in another animal there was a medullary cyst, and in the last mouse most of the ovary was enclosed in a cyst, which presumably was derived from the germinal epithelium. In the center there was a conglomerate duct. In the second oldest group of mice, there was in 1 animal, which had received an injection of potassium iodide, underneath the germinal epithelium, a collection of large ducts of germinal epithelial origin. There was also a conglomerate duct in the center, lined with cylindric epithelium; it may perhaps have been the continuation of a medullary duct. In addition there was in the medulla a large body consisting of large polyhedral cells not unlike liver cells. In a second mouse of this group there were some coiled ducts in the center of the ovary and in a third mouse a medullary cyst was seen. In the third group medullary cysts were noted in 2 mice; in one of these there was, in addition, in the hilus an angioma-like network of widely dilated capillaries which reached to the capsule of the cortex. Pressure exerted by the blood vessels destroyed the tissue situated between the dilated capillaries. In a third mouse of this group dilated lymph vessels were found in the medulla. In still another group of mice large cysts, probably of medullary origin, were found in 3 animals. In 1 breeding mouse invaginations of the germinal epithelium were seen. In still another animal an adenoma-like structure was present; it was derived from medullary ducts. In 2 other mice cysts were noted, lined with cylindric epithelium and surrounded by interstitial gland.

Period 3.—The interstitial gland consisted mainly of small or medium-sized cells derived probably from the theca interna. There was also much lutein-like tissue, which may have originated either in hemorrhagic corpora lutea or in interstitial gland that had changed into lutein-like tissue. Great numbers of largely hyalinized or gelatinized corpora lutea, which represented the greater portion of the ovaries in a number of mice, exerted pressure on the interstitial gland, which was thus reduced in size. Yellow interstitial gland tissue was not prominent. Hyaline material infiltrated the interstitial gland in places, while in other places there was extreme hyperemia in remnants of this tissue. In one ovary ducts seemed to give origin to interstitial gland.

Ducts originating in the germinal epithelium were numerous; there were also medullary ducts, and the latter could become converted into medullary cysts. Some ducts could connect with interstitial gland tissue, in particular also with lutein-like tissue, and in such cases again it appeared as if they gave origin to this tissue. Subgerminal spindle cell tissue accompanied ducts into the deeper parts of the ovary, and some of these ducts consisted of several layers of cells, which at first were solid but gradually seemed to develop into larger vacuolated or yellow cells. There were mitotic figures in some of the cells of the duct epithelium. In other places ductlike structures connected with tissue which was composed of large cells with large vesicular nuclei; the cytoplasm of some of these cells could be reduced in amount, and in this tissue mitotic figures also could be present. In 2 mice, 19 months old, adenoma-like structures developed; they seemed to have arisen from prolongations of the germinal epithelium, and they appeared to be composed, at least in some places, of conglomerated ducts. In one of the 2 mice these formations were present in both ovaries, and they reached into the interstices between gelatinized corpora lutea; they were covered by germinal epithelium and contained some large vessels.

Period 4.—In a 22 month old mouse almost all the ovary had become gelatinized, and the gelatinized area included apparently also the interstitial gland; there was only a small amount of typical interstitial gland left, and it contained a few vacuolar yellow cells. This observation suggests that hyalinized corpora lutea may be able to contribute to the formation of the interstitial gland. There was also

present a tumor-like structure consisting of coiled ducts containing mitotic figures; it was probably derived either from the germinal epithelium or from medullary ducts. A considerable part of the ovary was replaced by dilated capillaries, between which only some remnants of ovarian tissue were left. In a 21 month old mouse there was a corpus luteum-like structure which seemed to have given origin to interstitial gland. An artery in the tissue surrounding the ovary was ensheathed by dense hyaline tissue. In a 20 month old animal ductlike structures appeared to change to interstitial gland. There were nests of cells with vesicular nuclei, each of which contained a single central nucleolus. Most of the ovary consisted of interstitial glandlike tissue which seemed to have developed from corpora lutea, and a great part of this interstitial gland tissue was situated around vessels. In addition there were structures consisting of coiled ducts which could resemble corpus luteum tissue. In the breeding mouse small-celled interstitial gland tissue was seen.

Ducts penetrated from the germinal epithelium into the deeper ovarian tissue, and subgerminal spindle cell tissue reached a little into gelatinous lutein tissue. In 2 animals ducts gave rise to tumor-like structures, in one of which mitotic figures were present. In some instances it was difficult to decide whether ducts were of germinal epithelial or of medullary origin; this was particularly so in the case of the coiled tumor-like formations mentioned. As stated, some of these ductlike structures seemed to change into interstitial gland tissue. In the breeding mouse a large papillary cyst was seen; it was lined by cylindric epithelial cells. Blood vessels were present in the papillae which protruded into the cavity of the cyst, and the latter contained a colloid material. In this case, also, it was uncertain whether the cyst was of germinal epithelial or of medullary origin, and in this case also, the beginning of an excess formation of ducts was noted.

COMPARISON OF CHANGES IN INTERSTITIAL GLAND AND DUCTS TAKING
PLACE WITH INCREASING AGE IN STRAINS C, AKA, OLD BUFFALO,
NEW BUFFALO, C57 BLACK, C3H AND A WITH THOSE
NOTED IN STRAINS CBA AND D

I shall describe more briefly how far conditions found in various other strains agree with those described in strains CBA and D.

STRAIN C.—Only mice which were in periods 1, 2 and 3 of life were available; mice of period 4 were lacking. In the interstitial gland conditions were about the same as in that of strain CBA. In the main this tissue seemed to be derived from the theca interna of atretic follicles, but a participation of corpora lutea could not be excluded in every case.

Ducts were not conspicuous in period 1, and they were on the whole infrequent in most animals of this strain during periods 2 and 3.

STRAIN AKA.—In period 1 the interstitial gland was typical. In some instances it was arranged in strands between large capillaries in the medulla of the ovaries. With increasing age the size of the interstitial gland, as well as the number of yellow or brownish pigment cells, increased. In period 4 groups of yellow, often vacuolated cells were prominent, and some of the pigment seemed to have been derived from the hemoglobin of red blood corpuscles. In some packages of yellow cells the nuclei were lost. Ducts of germinal epithelial origin, as usual, were not noted in period 1; they began to become prominent only in period 2. In period 3 medullary ducts, also, were numerous. A medullary cyst exerted pressure on the ovary proper. In period 4, also, enlarged medullary ducts were seen, and some of the ducts in the deeper region of the cortex were filled with vacuolar

yellow cells; these ducts seemed to give origin to interstitial gland. There were also collections of ductlike structures, with or without lumens, lined with rather large, well developed cells, some of which had two nuclei. There were some mitotic figures in these collections of ducts, which resembled adenoma. It was difficult to decide whether these ductlike structures had actually been converted into interstitial gland. In the medulla there were some arteries whose muscularis was hyaline; there was also a thrombosed artery in process of fibrosis.

STRAIN OLD BUFFALO.—In principle the conditions were similar to those observed in the other strains; old age changes became prominent only in period 4. There was, however, a difference of interstitial gland in Old Buffalo and in other mice in so far as this tissue was larger in amount in the Old Buffalo mice and that there was here much yellow interstitial gland tissue. Also the germinal epithelial ducts as well as the medullary ducts could become very numerous. The cells lining the ducts in places enlarged, became vacuolar and yellow and connected with interstitial gland tissue consisting of lutein-like cells. Such conditions suggested strongly that ducts were transformed into interstitial gland tissue. Ducts could also become coiled or convoluted and give rise to complex structures which likewise seemed to change into interstitial gland. In the hilus of the ovary were collections of alveolar formations which consisted of cells rich in cytoplasm. Corpora lutea, in which there was some indication of hyalinization, as well as atretic follicles with remaining granulosa cells, seemed to become incorporated in places in the interstitial gland. The subgerminal spindle cell tissue often accompanied germinal epithelial ducts downward into the ovary; but these strands of spindle cell tissue could penetrate still farther into the ovary in places, and they surrounded groups of yellow or light vacuolar interstitial gland cells. Instead of spindle cell tissue, strands consisting of hyaline material penetrated into the ovary in other places; they separated groups of interstitial cells and could also surround capillaries and larger vessels. Very characteristic was the invasion of the fat tissue surrounding the ovary by ducts with accompanying lutein-like interstitial gland and also by coiled duct formations. This indicated that these structures had assumed tumor-like growth tendencies. Cysts on the whole were not common in this strain, but in period 4 some papillary cysts of germinal epithelial origin were found. In these papillae there were blood vessels surrounded by groups of yellow vacuolar cells with vesicular nuclei. As in other cases, here also the problem arose as to whether these ducts themselves gave origin to the lutein-like tissue and to the yellow interstitial gland tissue, or whether they merely invaded the preformed lutein-like tissue. All these changes, on the whole, were more prominent in the older than in the younger mice of period 4.

NEW BUFFALO STRAIN.—The interstitial gland showed the typical age changes. In periods 1 and 2 the interstitial gland had the character of a young tissue, although the size of some interstitial gland cells and the number of yellowish brown cells it contained were greater in period 2 than in period 1. In period 3 a notable change in the condition of the interstitial gland occurred. Its relative size increased, as well as the size of many constituent interstitial gland cells, which first became vacuolar and then acquired yellowish brown pigment. Blood pigment seemed to be contained in some of these cells, and this was in accord with the fact that hemorrhagic areas were seen in the cortex or the medulla of the ovary and that there were in places dilated blood vessels, which could give rise to hemorrhages, as well as lymph vessels filled with lymphocytes. Also some hyaline arteries or hyaline areas appeared at that time. In period 4 these changes progressed. Hyalinization of the intima and media of arteries became more frequent, and there

were occasionally also hyaline rings around capillaries. The development of yellowish brown cell areas likewise progressed, and they were seen in the center of groups of nonpigmented vacuolar cells, from which presumably they had taken their origin.

Ducts, on the whole, were scarce in periods 1 and 2, although they were somewhat more frequent in period 2. As in the interstitial gland, so also in the ducts a noticeable increase took place in the number of both types of ducts in period 3. Also, the layer of subgerminal spindle cells was well developed in the cortex. It resembled distinctly the corresponding tissue found in the cortex of the adrenal gland. In both adrenal gland and ovary, from the peripheral areas of the cortex the spindle cell tissue may grow downward into the deeper parts of these organs, and in the ovary it may reach between the strands of the interstitial gland, although such a downgrowth was rare in period 3. In certain instances the slit which is lined by germinal epithelium on top of the ovary enlarged into a cyst. In some animals, not in all, papillae protruded into such a cyst, and ducts developing from the germinal epithelium reached down into the ovary. In several cases not only medullary ducts were found, but also medullary cysts which developed from the ducts. There was thus an increase in the cyst formation in period 3. In this period there were pictures suggesting a transition of some cortical as well as of medullary ducts leading to small cell or larger cell, vacuolar or pigmented interstitial gland tissue.

In period 4, in the oldest mice, between 2 and 3 years of age, the number of cortical ducts could be much reduced, while in medulla and hilus there were large convoluted ducts, the lumens of which could be filled with epithelial cells. In younger mice of this period some germinal ducts formed alveoli which were filled with solid cells. Some medullary ducts were surrounded by hyaline tissue, and directly underneath the cortical germinal epithelium a hyaline membrane could be seen covering a layer of spindle cell connective tissue cells. In this strain the development of the cortical ducts was therefore deficient in the oldest mice, but there was again an increased formation of hyaline structures.

STRAIN C57 BLACK.—C57 black differs from other strains in these respects: The interstitial gland differentiates more rapidly. As early as in period 1 yellowish brown tissue is relatively strongly developed, and there is already noticeable in this tissue the tendency to form syncytia; as a result the cell membranes between adjoining cells which are involved in this process disappear. In the course of time it was found that in these syncytia the nuclei could arrange themselves in a radiating direction, so that rosette-like structures developed. In contrast to the interstitial gland, the production of ducts remains relatively rudimentary in these mice, and the changes of ducts into adenoma-like structures and apparently also into interstitial gland are not so pronounced as in the other strains, although in period 4 the formation of ducts and the secondary changes which may take place in the latter are pronounced. It is of interest that in the adrenal gland of strain C57 black the development of yellowish brown pigmented cells with rosette-like arrangement of the nuclei was especially pronounced. It seems therefore that this structural peculiarity is not altogether due to conditions confined to the ovaries but is to be ascribed to more generalized conditions which are active also in other organs.

It is perhaps owing to the strong development of the interstitial gland that the ducts do not find a chance to grow out in large numbers toward the center of the ovary. In the various periods one may note in addition the following findings: In period 1 interstitial gland tissue was present as early as 4 or 5 weeks of age, although it was as yet smaller in amount at this age. In mice younger than 6 weeks yellow cell strands were lacking, and in mice up to 3 months of age they

were smaller in size. As in the other strains, so also in C57 black the pigment in some of these cells seemed to be derived from hemoglobin, and this applies also to a number of the animals in the later periods of life. Some germinal epithelial ducts were present in a breeding mouse, and in the lumens of other ducts there were phagocytic cells containing blood pigment or erythrocytes. In period 1 as well as in period 2 the interstitial gland seemed to take its origin largely from the theca interna of atretic follicles, and only occasionally regressing corpora lutea appeared to take part in its formation. In some places the large interstitial gland cells developed perhaps in part from the membrana granulosa of atretic follicles, which under certain conditions seemed to give origin also to rosette formation.

In period 2 the large yellow cells or the syncytia were preferably situated around blood vessels, especially in the medulla. Hyaline material in large amounts could surround vessels entering the ovary. This hyaline substance was probably different from the hyaline that develops in great amounts in the corpora lutea in various strains but only to a slight extent in strain C57 black. In some places large interstitial gland cells seemed perhaps to develop from the granulosa of atretic follicles, which, as stated, may be able under certain conditions to give origin to rosette formations.

In period 3 it appeared that the groups of yellowish brown cells could in places develop from the smaller cuboidal cells. The differentiation of interstitial gland was especially pronounced in the medulla of the ovary where this tissue was oldest. Yellow cells were frequently situated around large blood or lymph vessels in the medulla. Ducts of medullary and germinal epithelial origin were more frequent in period 3, as well as cysts derived from medullary or from germinal epithelial ducts.

In period 4 the interstitial gland resembled the formations observed in the preceding periods, but in addition there was an indication that a part of the interstitial gland developed from ducts growing in the ovarian tissue. In some places strands of yellow interstitial gland cells were observed breaking through the ovary into the surrounding fat tissue. In the medulla hyaline vessels were seen. Germinal epithelial ducts were somewhat more frequent in this period and they likewise could penetrate into the fat tissue surrounding the ovary. In 1 mouse there was present in the fimbria an adenoma-like structure which seemed to have taken its origin either from the germ epithelium or from the epithelium of the fallopian tubes. Some of the cells of this structure were vacuolated. There occurred also in the ovary convolutions of ducts lined with vacuolar epithelium which in 1 case appeared to give origin to interstitial gland. Near the hilus of the ovary there were some medullary ducts lined with high epithelium or with several layers of epithelium. Underneath the germinal epithelium typical subgerminal spindle cell tissue was noted in some places, while in other places a layer of fibrillar connective tissue was found. Dilated blood capillaries in the outer region of the cortex could give origin to hemorrhages, and strands of subgerminal tissue penetrated into the coagulum. In this strain, as also in some other strains, masses of lymphocytes could destroy various parts of the ovary in some mice—an indication that a certain number of animals were probably affected by leukemia.

STRAIN C3H.—At the earlier periods the interstitial gland tissue consisted predominantly of small or medium-sized cells which seemed to develop especially around atretic follicles and to originate from the theca interna of these follicles; the latter, accordingly, could perhaps be included in places in the interstitial gland. It is not probable that remnants of corpora lutea took a significant part in the formation of interstitial gland tissue, inasmuch as they often undergo hyalinization in this strain and such hyalinized areas were not visible in the interstitial gland. How-

ever, hyaline vessel walls were seen in period 4. The formation of yellow interstitial gland tissue remained on the whole moderate, and phagocytosis of erythrocytes could here also contribute to the production of yellow pigment. The interstitial gland was especially prominent in the medulla where dilated lymph vessels could traverse it. Ducts were moderate in number in periods 2 and 3, but in period 4 large amounts of convoluted ducts could give rise to adenomatous areas consisting apparently of masses of alveoli. These alveoli were surrounded by subgerminal spindle cell tissue—an indication that these formations were derived from the germinal epithelium which gave origin to ducts. In places pressure exerted on the subgerminal strands of spindle cells by the underlying tissue caused the destruction of the spindle cell layer.

STRAIN A.—In periods 1 and 2 the interstitial gland showed the structure characteristic of young animals. Yellow tissue was seen only rarely, although more often in period 2 than in period 1. Areas of hyalinization which occurred in period 2 in the interstitial gland may perhaps have been due to admixture of hyaline corpora lutea. Even in period 1 the possibility that corpora lutea participated in the formation of the interstitial gland could not be entirely excluded. In the youngest mouse, 6 weeks of age, no typical interstitial gland was seen. In period 1 also a large hemangioma-like body was found at one end of the ovary. In another mouse there were dilated lymph vessels in the medulla. Only a small number of ducts, mainly of medullary origin, but exceptionally also derived from the germinal epithelium, were seen.

In period 2 a large medullary cyst appeared. In 1 case a ductlike structure seemed to give origin to lutein-like tissue; but usually such formations were seen only in later periods.

In period 3 the vacuolation and formation of large vacuolated yellow cell groups made some progress. Some hyalinization was observed in the interstitial gland as an indication that hyalinized corpora lutea may have contributed to its formation. In the membrana granulosa of atretic follicles vacuolation and advancing degenerative changes could be observed. In the hilus of the ovary hyalinized arteries were seen. At this period germinal epithelial ducts were on the whole more frequent, although they were not prominent in all the mice. The slits lined with germinal epithelium and covering the ovaries in some cases could become transformed into cysts. Also medullary ducts lined with cylindric cells and containing cytoplasmic granules were observed.

In period 4 the interstitial gland underwent changes similar to those observed in other strains in older mice. Areas with large, yellow, vacuolated lutein-like cells, lining ducts, increased in size. These cells could exert phagocytic activity toward erythrocytes. In places dilated blood vessels gave rise to hemorrhages enclosed in cystlike formations in the interstitial gland tissue. Mitotic figures could occur in the vacuolar interstitial gland cells. The media and the intima of some vessels were hyalinized. The layer surrounding the arterial endothelium was infiltrated in various places with red corpuscles, so that these areas assumed a red stain with eosin. Also arteries seen in the tissue surrounding the ovary could be strongly hyalinized. Hyalinized and gelatinized areas in the interstitial gland suggested the inclusion of hyalinized corpora lutea in this tissue, but in addition very large follicles could be seen in which the outside cells, apparently corresponding to theca interna cells, were vacuolar and the inside cells, representing presumably granulosa cells, were lutein-like. It appears probable that the interstitial gland was derived at least partly from theca interna cells which had undergone corresponding changes. Germinal epithelial and medullary ducts, some of which were convoluted, could be lined with ordinary vacuolar cells or with

yellow vacuolar cells, and such cells could also fill the lumens of the ducts. Mitotic figures were seen in the cells of these ducts. It appeared as though these ducts gave origin to the lutein-like interstitial gland tissue. In several mice the slits lined with germinal epithelium enlarged into cysts which could be filled with blood. These hemorrhages resulted from the presence of much dilated vessels near the cysts. These cysts could be surrounded by subgerminal spindle cell tissue in accordance with their origin from germinal epithelium. One sees, then, in strain A changes in the interstitial gland which in principle are similar to those seen in other strains.

COMMENT

I shall now compare the variations which occur (1) in the interstitial gland structures and (2) in the ducts in the various strains of mice with increasing age.

Origin of the Interstitial Gland and Variations in the Amount of Interstitial Gland Tissue.—In general, and this applies with some reservations to all the strains, the interstitial gland increases in size with increasing age of the mice. In very young animals, 3 to 5 or 6 weeks old, the interstitial gland was as yet very rudimentary or lacking almost entirely. However, in strain C57 black in which this tissue shows a more rapid development and differentiation and more pronounced changes with old age than in some other strains, interstitial gland was already present in mice 4 to 5 weeks old. In periods 1 and 2 it consisted mainly of small and medium-sized cuboidal cells, but it was found in somewhat larger quantity in period 2 than in period 1. It underwent more marked changes in period 3 and especially in period 4. At this time it usually increased in size; the individual cells enlarged, and they tended to show more or less vacuolation and to accumulate yellow pigment. The largest quantity of the interstitial gland was often found in the medulla, where it inclined to be arranged especially around the larger vessels. As to its origin, it seems to be derived, at least to a considerable extent, from the theca interna of atretic follicles; but there are indications that corpora lutea, also, and even the membrana granulosa of atretic follicles may perhaps take part in its formation, as I shall discuss later. Variations, besides those already mentioned as occurring in strain C57, may depend on the prominence of the corpora lutea. Thus, especially in strain D, during periods 3 and 4, hyalinized and gelatinous corpora lutea were prominent. They could press on the interstitial gland, which was thereby diminished in amount. On the other hand, if the interstitial gland was strongly developed, it also could press on adjoining tissues and cause their destruction. Thus in strain C3H it was observed that pressure exerted by the interstitial gland on the subgerminal spindle cell tissue led in places to the destruction of the latter.

Origin and Relative Quantity of the Vacuolar and Yellow Interstitial Gland Tissue.—In general, yellow cells, which usually are vacuolar and

larger than the young cuboidal interstitial gland cells, are present only in small quantities in the interstitial gland in period 1 or they may be absent at that time. They increase somewhat in period 2. In strain CBA yellow pigment seemed to appear also in the membrana granulosa of atretic follicles. In strain D, in period 2 the yellow interstitial gland cells were situated especially around vessels in the medulla. In strain C57 there was yellow interstitial gland tissue present in period 1, and syncytia formed in this tissue as a result of the coalescence of neighboring cells. The nuclei could be arranged in the form of rosettes. In mice younger than 6 weeks yellow cell strands could already be seen, but from this time on up to the age of 3 months they were smaller in size. Also in the adrenal gland of strain C57 yellow cells, syncytia and rosettes were present. In regard to the yellow tissue we have therefore to deal with a peculiarity which is not limited to the ovaries. In strains C3H and A yellow tissue is rare in periods 1 and 2. In periods 3 and 4 it increased in all the strains, although the pressure exerted by hyaline corpora lutea may destroy yellow tissue, as, for instance, in some of the mice of strain D. In strain AKA there occurred patches of yellow tissue in which the nuclei had been lost. In the New Buffalo strain it could be distinctly seen that the yellow cells may develop in the center of groups of light vacuolar cells which presumably gave origin to the pigmented cells. They may also be derived from smaller cuboidal interstitial gland cells. In strain A there were atretic follicles in which the theca interna cells were vacuolar and the membrana granulosa lutein-like.

Dilatation of Vessels, Hemorrhages and Hemorrhagic Pigment in the Interstitial Gland.—The yellowish brown pigment which develops in the course of time in the aging interstitial gland cells is presumably chemically similar to or identical with the endogenous pigment present in the corpora lutea. However, there may be admixed to this pigment in places yellowish hematogenous pigment, which some of the larger vacuolated interstitial gland cells seem to take up from the surrounding tissue. There are sufficient opportunities for the formation of such hematogenous pigment in various places in the ovary and especially also in the interstitial gland tissue. Not rarely one sees many enlarged blood vessels in which the slowing down of the blood stream provides an opportunity for the red blood corpuscles to escape from the vessels or for larger hemorrhages to occur in the interstitial gland. Thus in strain CBA yellow cells seem to contain some blood pigment in period 4. The sources of the extraneous blood pigment were ovarian, especially medullary hemorrhages and hemorrhagic cysts. There were dilated capillaries in different parts of the ovary. But conditions of this kind could be noted also in early periods. Thus in strain D enlarged

medullary vessels were found in period 1. In period 2 some blood pigment was present in vacuolar cells, which had presumably taken it up from the outside by phagocytosis. In the hilus an angioma-like network of vessels was found; in addition dilated capillaries and lymph vessels occurred in adenoma-like structures. Also in period 3 hyperemia could be noted in the interstitial gland. A heavy angioma-like body was found likewise in strain A in period 1. In period 4 a hemorrhagic cyst was observed in the interstitial gland. In addition a germinal cyst was hemorrhagic; the source of the hemorrhage in this instance was probably some dilated vessel surrounding the cyst. Similar dilatation of vessels and hemorrhages were found also in other strains, mainly in periods 3 and 4; but hematogenous pigment could be seen even as early as in period 1. In strain D there was observed in period 2 a body of polyhedral cells, the nature and origin of which could not be determined.

Relation of Corpora Lutea and Atretic Follicles to the Interstitial Gland.—There are strong indications that the theca interna of atretic follicles is one of the principal sources of the interstitial gland, but the membrana granulosa of atretic follicles, also, perhaps, takes part in the formation of the interstitial gland in some instances. Thus in strain C57 it seems that in the stage of atresia the granulosa, which may participate also in the rosette formation, may be included in interstitial gland tissue. In strain A the granulosa of atretic follicles seemed to undergo lutein-like changes and then to become incorporated in the interstitial gland. Moreover, in various strains pictures have been seen which suggest that regressing corpora lutea as well may become parts of the interstitial gland. A strong argument in favor of this view is the observation that occasionally areas of hyalinization are seen in the interstitial gland in strains in which the corpora lutea undergo hyaline or gelatinous degeneration. This was the case, for instance, in strain D in period 3. It was noticeable, too, in strain A during period 4. On the other hand, in strain C3H, in which hyalinization likewise occurs in corpora lutea, the interstitial gland was found free of such changes. In some respects one might therefore regard the interstitial gland as a reservoir into which various kinds of regressing or degenerative constituents of the ovary are pushed and deposited until in the end they disappear.

Change in Duct Formation with Increasing Age in Different Strains of Mice.—Two kinds of ducts have to be distinguished: (1) those that develop by growing out from the remnants of the wolffian body in the medulla; (2) those that develop from the germinal epithelium covering the surface of the ovary by growing downward. The latter can be distinguished from the former in many cases by the fact that the subgerminal spindle cell tissue surrounds them in their downgrowth and

furnishes a lining for these germinal epithelial ducts. Another means of differentiation may be that at least in some instances the epithelial cells lining the medullary ducts may be higher, cylindric, in contrast to the flat cuboidal epithelium of the germinal epithelial ducts. In general the number of ducts which form increases with increasing age, and in addition various changes may take place in them. Thus the cells lining the ducts may become vacuolated, acquire yellow pigment and fill the lumens of the ducts. Or the ducts may become coiled, convoluted, and form adenoma-like structures, which on cross section may appear as a collection of alveoli. When these ducts come into contact with interstitial gland or with corpus luteum tissue, one gains the impression in many places that one has to deal not only with a contact between ducts and interstitial gland or lutein-like tissue but with an actual tendency of the ducts to transform into interstitial gland. In the ducts and in this real or apparent transitional tissue between ducts and interstitial gland, mitotic figures may occasionally appear as an indication of the growth processes which can take place in these tissues. All these changes tend to occur preferably in older mice during period 3 and especially during period 4. However, some uncertainty exists as to the interpretation of these pictures. Instead of an actual transformation of ducts one may have to deal merely with contact between interstitial gland and ducts in which the cells lining the ducts can become similar to interstitial gland cells. Moreover, in some instances one may have to deal not with real ducts but merely with imitation ducts. This appearance may be due to the growing down of the subcapsular spindle cells into the interstitial gland or lutein-like tissue. The spindle cells may surround strands of interstitial gland tissue, which thus may give the impression of ducts. Such an occurrence also might produce pictures simulating a transition between ducts and interstitial gland tissue.

In rather rare instances convoluted ducts may appear as early as in period 2. This occurred, for instance, in a mouse belonging to strain D. Also an adenoma-like structure developed in strain D in period 2. Furthermore, in a mouse of strain D, in period 2, medullary ducts were lined with cells which resembled those seen in ordinary sebaceous glands, and in general similar changes may take place in germinal epithelial ducts and in medullary ducts. Both germinal epithelial and medullary ducts may coil and subsequently present the appearance of adenoma. In all the strains the number of ducts increased and the usual further changes took place during periods 3 and 4.

Formation of Cysts.—There were two kinds of cysts, as there were two kinds of ducts, in the ovary: medullary cysts and epithelial germinal cysts. The former were derived from medullary epithelial structures, and they could be present as early as in periods 1 and 2, although this was not the usual occurrence. The germinal epithelial cysts were

derived as a rule from the germinal epithelial slits surrounding the ovary, which became dilated. The epithelium covering the ovary directly was usually higher than the epithelium lining the outer wall of the cyst, which was pressed flat. As stated, these cysts are not usually present in periods 1 and 2, although they did occur in some cases; for instance, in strain D a germinal epithelial cyst was noted in period 2. In strain A there was in period 4 a cyst filled with blood which was probably derived from dilated hyperemic vessels surrounding the cyst. Papillae consisting of central vessels surrounded by lutein-like interstitial gland tissue could protrude into medullary as well as into germinal cysts. Medullary cysts in places exerted pressure on the remainder of the ovary.

Transitions Between Ducts or Adenoma-like Structures and Interstitial Gland Tissue.—There is a sequence of changes which the ducts may undergo with the advancing age of the host. They first may coil and give rise to convolutions, which then can become adenoma-like. In the end either the ducts or the adenoma-like tissue may perhaps change to interstitial gland or to an intermediate lutein-like tissue. I have discussed already these apparent changes, as well as those which may take place in the ducts themselves, and have mentioned the differences in the interpretation of these changes. These alterations take place mainly in period 4 or to a more limited extent in period 3. There was one exception to this rule: In strain A a ductlike structure seemed to give origin to interstitial gland as early as in period 2.

Behavior of Subgerminal Spindle Cell Tissue.—Underneath the germinal epithelium a layer of spindle cell tissue is found. This tissue differs from resting spindle cell connective tissue by the great cellularity of the layer and by the absence or rareness of fibrils between the cells. The tissue resembles a similar layer found underneath the capsule in the adrenal gland of mice. In both adrenal glands and ovaries this tissue may send strands downward into the deeper structures of these glands, and during this process it may gradually become richer in connective tissue fibrils and thus may come to resemble ordinary connective tissue. In the adrenal cortex the subcapsular spindle cell tissue sends strands of spindle cells between the rows of cortical tissue as far down as the intermediate zone separating cortex and medulla. In the ovary the spindle cell tissue often envelops the ducts which develop from the germinal epithelium, and it envelops also parts of the germinal epithelial cysts; or the spindle cells may grow downward into the interstitial gland, thereby separating strands of interstitial gland cells from one another. In strain D the subgerminal spindle cell tissue was seen to penetrate also into gelatinous corpora lutea.

Mitotic Proliferation in Interstitial Gland and Ducts.—Mitotic figures appeared in various strains in structures in which they usually were not seen in mice. For instance, in strain CBA they were noted in period 4 in coiled ducts either of germinal epithelial or of medullary origin, also in transitional lutein-like interstitial gland tissue which may perhaps have been derived from these ducts. In strain D mitotic figures likewise were noted in ducts and in transitional tissue during period 3. In period 4 they appeared in adenoma-like structures derived from ducts and also in ducts of germinal epithelial and of medullary origin. In strain A they were noted in ducts and interstitial gland tissue during period 4. They were thus mostly seen in period 4, instead of in younger mice, but they did appear also in period 3; they seemed to be the expression of the increased activity of these structures in older mice and were perhaps connected with the apparent transition between ducts and interstitial gland tissue.

Penetration of Ducts and Interstitial Gland Tissue into the Fat Tissue Surrounding the Ovary.—In three strains it was observed that ducts and interstitial gland tissue, the latter sometimes assuming a lutein-like appearance, were able to penetrate through the surface of the ovary into the surrounding fat tissue. In every case this was observed only during period 4, therefore in old mice, and it was evidently related to the frequent abnormally strong development of these structures in mice of advanced age. In strain CBA germinal ducts which were coiled penetrated in some places into the surrounding fat tissue. Also in strain Old Buffalo ducts, lined with lightly staining vacuolar cells or with large yellow vacuolar cells, and in particular coiled ducts, including germinal ducts surrounded by lutein-like tissue, invaded the surrounding fat tissue. In strain C3H germinal ducts together with yellow interstitial gland tissue broke through the surface of the ovary into the surrounding fat tissue. In this connection mention might be made of another peculiarity which was noted in strain CBA during period 4, namely, the epithelial pearls which developed in ducts and especially in cystically dilated ducts, while other ducts were lined with flat epithelium. The pearls consisted of squamous epithelium surrounding concentric hyaline material. The changes mentioned in this paragraph and also in the following paragraph resembled very much conditions which may be seen during the formation and growth of tumors.

Hyalinization of Structures Other Than Corpora Lutea in the Ovaries of Mice During Various Periods of Life.—A continuous increase of hyalinization takes place in the successive periods of life. Hyalinization is as yet slight in period 1; it was observed in only a few mice in strain A in this period. It became much more widespread during periods 2 and 3. There was probably an increase in period 3 as compared with period 2, although the difference between these two

periods was not pronounced. It definitely increased in period 4 over periods 2 and 3. While the number of mice available for study at that time of life was relatively small in all the strains, the proportion of mice affected by hyalinization was greater during period 4 than during periods 2 and 3. Therefore with increasing age of mice the tendency toward hyalinization increases. In this respect conditions in the mouse correspond to those noted in the human species. There is a relation between the processes of hyalinization which take place in the corpora lutea and those in or around vessel walls. As we have seen, in corpora lutea hyalinization may begin around vessels, which then may be surrounded by a hyaline ring. But hyalinization of vessels does not have to be limited to the corpora lutea; it may occur also in the ovarian tissue outside the corpora lutea, and even the arteries in the tissue surrounding the ovaries may be affected by this process. However, that an actual connection exists between the hyalinization of vessels and that of corpora lutea, at least in some cases, is indicated also by the fact that in period 1 hyaline vessels were found only in strain A, in which the tendency to hyalinization of corpora lutea is strong. On the other hand, in many instances processes of hyalinization may appear in or around vessel walls even without the presence of hyalinized corpora lutea, and in strains in which hyalinization of corpora lutea is rare. Furthermore, hyalinization may affect structures other than corpora lutea. It is therefore probable that hyalinization of vessel walls may occur independently of hyalinization of corpora lutea but that in some instances this process occurring in corpora lutea may be associated with similar changes in or around the vessel walls. In sections stained with hematoxylin and eosin no difference can be detected between the hyaline deposits in vessel walls and those in corpora lutea. Differential staining of amyloid substance may not succeed in the ovary of the mouse, as indicated in earlier experiments, in which an attempt was made to differentiate amyloid from other hyaline substances in the adrenal gland of the mouse.

As stated already, hyalin may be deposited either around a vessel or in the media or the intima of the vessel. It was especially during period 4 in older mice that hyaline substance was discovered in the intima underneath the endothelium or in the media of the vessel in addition to perivascular hyaline substance. Hyaline material appeared not only around arteries but also around capillaries. In some cases the vessel walls could undergo more acute injury leading to necrosis, with or without the necrotic areas being subsequently incrustated with calcium salts. This occurred especially in vessels which were surrounded by hyaline-gelatinous corpus luteum tissue, which presumably exerted pressure on the walls of the vessels and thus interfered with their supply of oxygen or of foodstuffs. Such necrotic areas, on the

whole, were not frequent, but they were observed in period 2 as well as in period 3. While hyalinization was therefore quite common in arteries, the farther going changes, consisting in gelatinization and solution, which are so frequent in corpora lutea, seemed to be lacking in the vessel walls, which underwent in the mouse changes similar to those seen in the other species.

As stated processes of hyalinization were not limited to corpora lutea and blood vessels but affected also certain other structures. Thus in follicular atresia a hyaline membrane could be seen separating membrana granulosa and theca interna. Furthermore, instead of a layer of spindle cells lying underneath the germinal epithelium, this cellular layer could in places be replaced by a hyaline membrane. Likewise, instead of strands of spindle cells, extending from the subgerminal area downward, layers of hyaline material passed downward into the interstitial gland tissue in some instances and separated areas of interstitial gland from each other. Not only the vessel walls were surrounded by streaks of hyaline tissue but also ducts which develop in the ovaries with advancing age and in particular the medullary ducts. Lastly there may appear in ovaries in different places diffuse areas of dense hyaline material. They may be seen in various parts of the ovary, but perhaps they are more frequent in the medullary portion than elsewhere. These areas do not seem to be connected with the corpora lutea; they may perhaps develop around blood vessels, although this is not certain, at least not in all cases. Again, nutritional deficiencies may lead to necrosis in certain parts of the hyaline material, and such areas were surrounded by foreign body giant cells in some instances.

As to the mode of formation of these various hyaline deposits, the frequency with which they are observed around arterial walls suggests that they may be due to the deposition of material carried by the blood and passing from the blood vessels into the surrounding tissue, where it perhaps undergoes further chemical or physical changes. However, this can hardly be taken to be the only mode of development of these hyaline structures if one considers the fact that hyalin is also deposited around ovarian ducts and in other sites where it cannot well represent a modified transudate passing through the vessel wall to the outside. Instead in some instances it may perhaps be due to a hyaline transformation of connective tissue fibers.

In regard to the question whether a strain could be genetically predisposed to hyalinization outside of that seen in corpora lutea, it is significant that no hyalinization was noted in strain C. Hyalinization was rare also in strain CBA, but it appeared in this strain in period 4. In strain C57 it occurred in periods 2, 3 and 4, although the corpora lutea showed only a slight tendency to hyalinize. While in the case of this strain there was apparently no parallelism between hyalinization

of corpora lutea and that of other structures, in general in strains which exhibited a greater tendency toward hyalinization of the corpora lutea, there seemed also to be a greater tendency toward hyalinization of other structures. Thus in strains AKA and D and also in strain C3H there was generally much hyalinization of both kinds, whereas in strain C and in strain CBA there was only a slight tendency toward both these types of hyalinization. However, the data available so far make possible only tentative conclusions in this respect.

SUMMARY

In all the strains of mice the interstitial gland of the ovary increased in size with the increasing age of the mice; in very young mice it was as yet rudimentary. The interstitial gland cells underwent marked changes especially in periods 3 and 4, when the cells tended to become more or less vacuolated and to accumulate yellow or brown pigment. The interstitial gland tissue seemed to be derived mainly from the theca interna of atretic follicles, but there were indications that regressing corpora lutea, also, or even the membrana granulosa of atretic follicles could be incorporated in the interstitial gland. The fact that hyaline changes occurred in the interstitial gland in strains in which similar changes took place in corpora lutea also supports the view that regressing corpora lutea may become a part of the interstitial gland. In strain C57 black the interstitial gland had a tendency to form syncytia and rosettes, and the pigment in this tissue developed at an early stage; similar changes occurred in the adrenal gland of strain C57 black. In some strains the corpora lutea, especially the hyalinized ones, exerted pressure on the interstitial gland and helped to destroy it.

There occurred mainly in periods 3 and 4, but sometimes as early as in periods 1 and 2, hemorrhages in the ovaries, especially in the interstitial gland; these occasionally were enclosed in cystlike spaces and were associated with much dilated capillaries. The vacuolated interstitial gland cells could take up red blood cells or hematogenous pigment. In 1 case the presence of dilated vessels adjacent to a germinal epithelial cyst seemed to have resulted in the filling of the cyst with coagulated blood. Also hemangioma-like structures were found in periods 1 and 2 in strains A and D.

With advancing age the germinal epithelial and the medullary ducts increased in number. As a rule, they became very numerous in periods 3 and 4. In period 3, and still more so in period 4, the ducts underwent further changes, particularly coiling, formation of adenoma-like structures, vacuolation of duct cells and apparent or real transitions leading to lutein-like interstitial gland tissue. Occasionally an adenoma-like structure was found as early as in period 2. Cysts also increased in number with increasing age, although some cysts, especially those of

medullary origin, were found in periods 1 and 2. The medullary cysts were derived from medullary ducts, and the germinal epithelial cysts developed as a result of dilatation of the germinal epithelial slits covering the ovary.

The layer of subgerminal spindle cell tissue corresponding to similar tissue in the subcapsular layer of the adrenal gland formed a sheet around germinal epithelial ducts and cysts and thus helped to distinguish these structures from ducts and cysts of medullary origin. These spindle cells also were most prominent in period 4, but they are noticeable at earlier times.

With increasing age, mainly in period 4 but to some extent also in period 3, certain additional growth phenomena were seen which were not unlike changes taking place in early tumor formation. Mitotic figures appeared in medullary as well as in germinal epithelial ducts, in adenoma-like structures, in lutein-like tissue and in fully developed interstitial gland tissue. These tumor-like processes have been seen so far only in some of the strains. Ducts accompanied by lutein-like interstitial gland or perhaps interstitial gland alone could penetrate through the surface of the ovary into the surrounding fat tissue.

Processes of hyalinization occurred not only in or around arteries and capillaries but also in various other structures of the ovaries. They increased in frequency with increasing age and were partly associated with processes of hyalinization which took place in corpora lutea. It is probable that the hyaline changes which were noted around blood vessels and in the walls of blood vessels were of the same kind as those which occur in blood vessels generally with increasing age.

EFFECTS OF FOLIC ACID ANTAGONISTS INOCULATED IN EMBRYONATED EGGS

PHILIP F. WAGLEY, M.D.*
AND
HERBERT R. MORGAN, M.D.†
BOSTON

THE ANTAGONISTS of folic acid that have been most carefully studied are analogues of four main types. In the first type the substituents of the pteridine ring are altered. In the second a substituent is introduced on the amino group of the para-aminobenzoylglutamic acid portion of the molecule. In the third a different amino acid is substituted for glutamic acid. Combinations of these variations compose the fourth type.

The following observations are concerned with the effects on chick embryos of three folic acid antagonists belonging to types 1, 2, and 4. These studies were made by a new technic for evaluating the hemopoietic activity of various substances in the chick embryo. Some of the findings have appeared in a preliminary note.¹

MATERIALS AND METHODS

Chick embryos 6 to 8 days old were inoculated via the yolk sac with three folic acid antagonists (4-aminopteroylglutamic acid, N¹⁰-methylpteroic acid, (methyl-4-aminopteroylglutamic acid) alone or with folic acid,² liver injection or vitamin B₁₂³ in varying amounts. All the substances except injection U.S.P. (solution liver extract purified,[®] 15 U.S.P. units per cubic centimeter) were dissolved in distilled water. The embryos were incubated at 35C. All deaths occurring in the first forty-eight hours were considered post-traumatic and discarded from the study.

* Research Fellow of the American College of Physicians.

† Senior Fellow in Medical Sciences, National Research Council.

This investigation was aided by a grant from the National Foundation for Infantile Paralysis.

From the Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard), Boston City Hospital, and the Department of Medicine, Harvard Medical School.

1. Wagley, P. F., and Morgan, H. R.: *Bull. Johns Hopkins Hosp.* **83**:275-278, 1948.

2. The 4-aminopteroylglutamic acid (lot 7-7843C), N¹⁰-methylpteroic acid (lot 7-7623A), methyl-4-aminopteroylglutamic acid (lot 7-8185) and folic acid (as synthetic pteroylglutamic acid) were obtained through the Lederle Laboratories, Pearl River, N. Y.

3. Vitamin B₁₂ was provided by Dr. A. Gibson, of Merck & Co., Inc., Rahway, N. J.

Blood was obtained for cell counts and hemoglobin determinations by removing the shell over a large allantoic vessel previously visualized by transillumination. The shell membrane was kept intact. A drop of liquid petrolatum was placed on the intact membrane to improve visualization. Venipuncture was done with a 27 gage needle and a 0.25 cc. or 0.5 cc. syringe. Potassium-ammonium oxalate mixtures added to such blood did not prevent a coagulum from forming. Cell counts were therefore made immediately, using isotonic sodium chloride solution or Gower's solution as a diluent. Hemoglobin determinations were made with an Evelyn colorimeter.

Yolk sacs were fixed in 10 per cent acetic acid-Zenker's solution, and microscopic sections were stained with eosin-methylene blue.⁴

TABLE 1.—*Effect of 4-Aminopteroylglutamic Acid on Chick Embryos*

Series	4-Amino pteroyl- glutamic Acid, Mg.	Survival of Embryos, Age in Days								Microscopic Appearance of Yolk Sac Blood Islets
		7	8	9	10	11	12	13	14	
1	1 0	3/3*	3/3	3/3	0/3	
2	0 1	4/4	4/4	4/4	2/4	0/4		
3	0 01	5/5	5/5	5/5	5/5	2/5	0/5	.	. .	Diminution in size and number of blood islets; pyknosis, karyolysis and karyorrhexis of nuclei
4	0 005	10/10	10/10	10/10	10/10	7/10	6/10	4/10	0/10	Diminution in size and number of blood islets; pyknosis, karyolysis and karyorrhexis of nuclei
5	0 001	10/10	10/10	10/10	10/10	10/10	10/10	8/10	7/10	Diminution in size and number of blood islets; pyknosis, karyolysis and karyorrhexis of nuclei
6	Distilled water 1 0 cc	5/5	5/5	5/5	5/5	5/5	3/5	3/5	1/5	Normal

* The numerator is the number of embryos that survived; the denominator, the number given injections.

† Two embryos were killed for study. The average cell count and hemoglobin determination are given in the text.

EXPERIMENTS

EXPERIMENT 1—Five series of 7 day chick embryos were inoculated with various amounts of 4-aminopteroylglutamic acid. A sixth series received only an inoculum of distilled water as a control. The observations on survival and the microscopic observations on the blood-forming tissues are summarized in table 1.

There appeared to be a shortening of the survival time with an increase of the amount of the antagonist. In addition, histologic study of the blood islets of the yolk sac revealed a decrease in their number, size and cellularity. The hemopoietic cells that were still present showed marked pyknosis and fragmentation of the nuclei. Other nuclei showed decreased basophilia. These changes are illustrated in figure 1, which shows a blood islet of the yolk sac of a 12 day old chick embryo which had been inoculated on the seventh day of life with 0.005 mg. of 4-aminopteroylglutamic acid. For comparison, a normal

4 Miss Lillian M. Leavitt, of the Mallory Institute of Pathology, prepared the microscopic sections

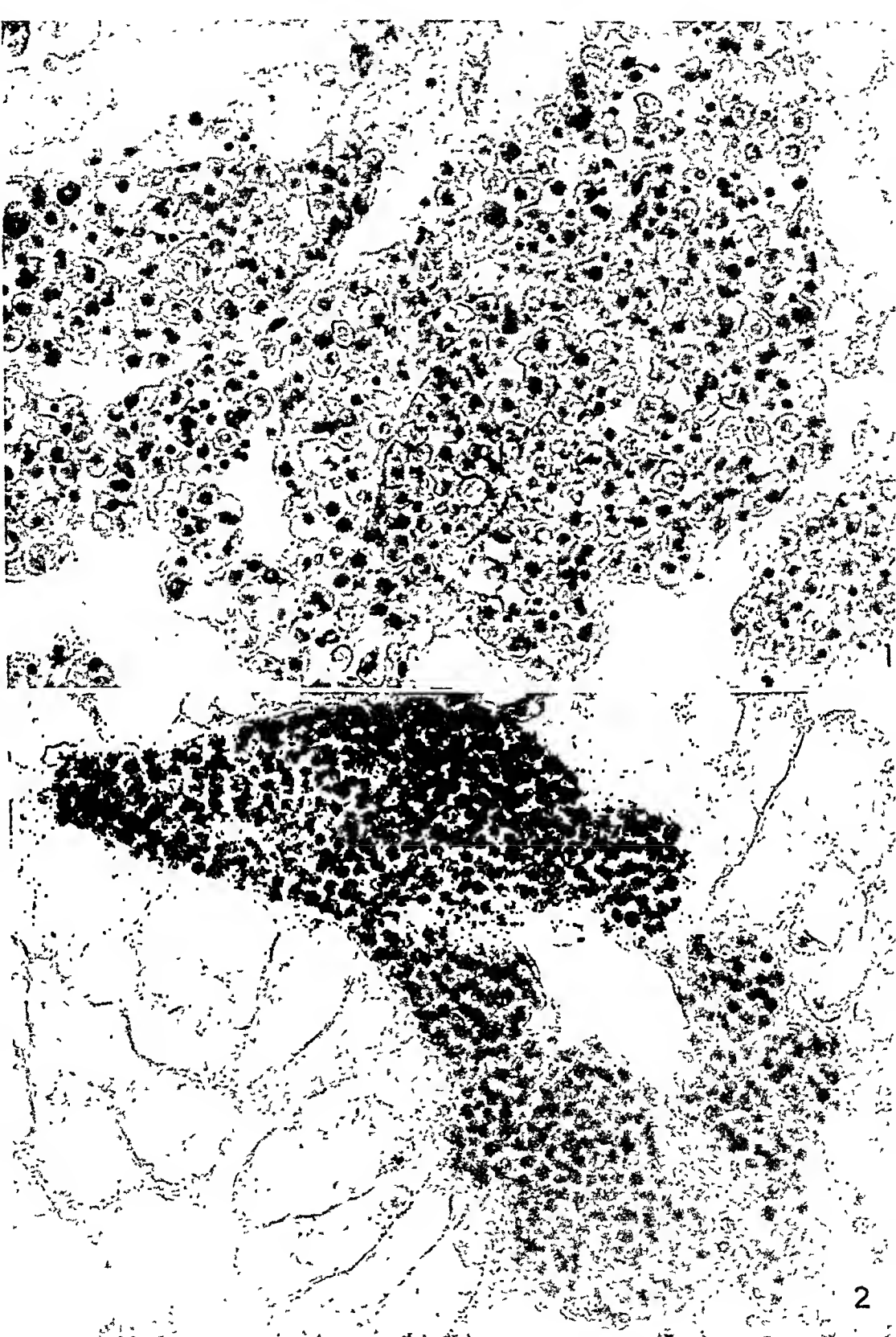


Fig. 1.—Yolk sac blood islet of a 12 day old chick embryo inoculated five days previously with 0.01 mg. of 4-aminopteroylglutamic acid. There is marked loss of cellularity (many of the cells visible are intravascular) with pyknosis and karyolysis of the nuclei. $\times 333$.

Fig. 2.—Yolk sac blood islet of a normal 12 day old chick embryo. $\times 333$.

yolk sac blood islet of a 12 day chick embryo of the control group of this experiment (series 6) is shown in figure 2.

Cell counts and hemoglobin determinations were made on the blood of 2 embryos of series 4 and 2 embryos of series 6 at the age of 13 days. The total blood cell counts of the 2 receiving 0.005 mg. of 4-aminopteroylglutamic acid six days previously (series 4) averaged 1,300,000 per cubic millimeter, and the hemoglobin levels averaged 5.1 Gm per hundred cubic centimeters of blood. The corresponding values obtained on the same day in the controls (series 6) aver-

TABLE 2.—*Influence of Certain Hemopoietic Substances on the Effects of 4-Aminopteroylglutamic Acid in Chick Embryos*

Series	Drug	Dose	Age at Inoculation in Days	Survival of Embryos, Age in Days								Microscopic Appearance of Yolk Sac Blood Islets
				7	8	9	10	11	12	13	14	
1	4-aminopteroylglutamic acid	0.01 mg.	7	10/10†	10/10	10/10	9/10	6*/10	6/10	6/10	5/10	Slight diminution in cellularity of blood islets
	Folic acid	12.5 mg.	6									
	Liver injection U. S. P.	3.75 units	6									
2	4-aminopteroylglutamic acid	0.01 mg.	7	9/9	9/9	9/9	9/9	6*/9	6/9	6/9	6/9	Slight diminution in cellularity of blood islets
	Folic acid	12.5 mg.	6									
3	4-aminopteroylglutamic acid	0.01 mg.	7	12/12	12/12	12/12	9/12	5*/12	2/12	2/12	1/12	Diminution in size and number of blood islets; pyknosis, karyolysis and karyorrhexis of nuclei
	Liver injection U. S. P.	3.75 units	6									
4	4-aminopteroylglutamic acid	0.005 mg.	8	3/3	3/3	3/3	3/3	2/3	2/3	0/3	Diminution in size and number of blood islets; pyknosis, karyolysis and karyorrhexis of nuclei
	Vitamin B ₁₂	0.005 mg.	8									
5	4-aminopteroylglutamic acid	0.005 mg.	8	1/1	1/1	1/1	1/1	1/1	1/1	1/1	Diminution in size and number of blood islets; pyknosis, karyolysis and karyorrhexis of nuclei
	Vitamin B ₁₂	0.011 mg.	8									
6	4-aminopteroylglutamic acid	0.01 mg.	7	11/11	11/11	11/11	10/11	5*/11	5/11	2/11	0/11	Diminution in size and number of blood islets; pyknosis, karyolysis and karyorrhexis of nuclei

* Three embryos were killed for study.

† The numerator is the number of embryos that survived; the denominator, the number injected.

aged 1,800,000 per cubic millimeter and 6.5 Gm. per hundred cubic centimeters of blood.

About 0.01 to 0.005 mg. of 4-aminopteroylglutamic acid seemed to be the smallest amount consistently giving detectable alteration in the survival time and this histologic appearance of the blood islets under the conditions of these experiments.

EXPERIMENT 2.—The purpose of experiment 2 was to determine whether or not folic acid, liver injection U.S.P. (solution liver extract, purified,[®] 15 U.S.P. units per cubic centimeter) or vitamin B₁₂ would protect chick embryos from the effects of 4-aminopteroylglutamic acid. As shown in table 2, 6 series of embryonated eggs 7 to 8 days of age were inoculated with 0.01 or 0.005 mg. of the folic acid antagonist. In series 1, 2 and 3 the embryos were inoculated with liver injection U. S. P. and folic acid, in the amounts indicated, twenty-

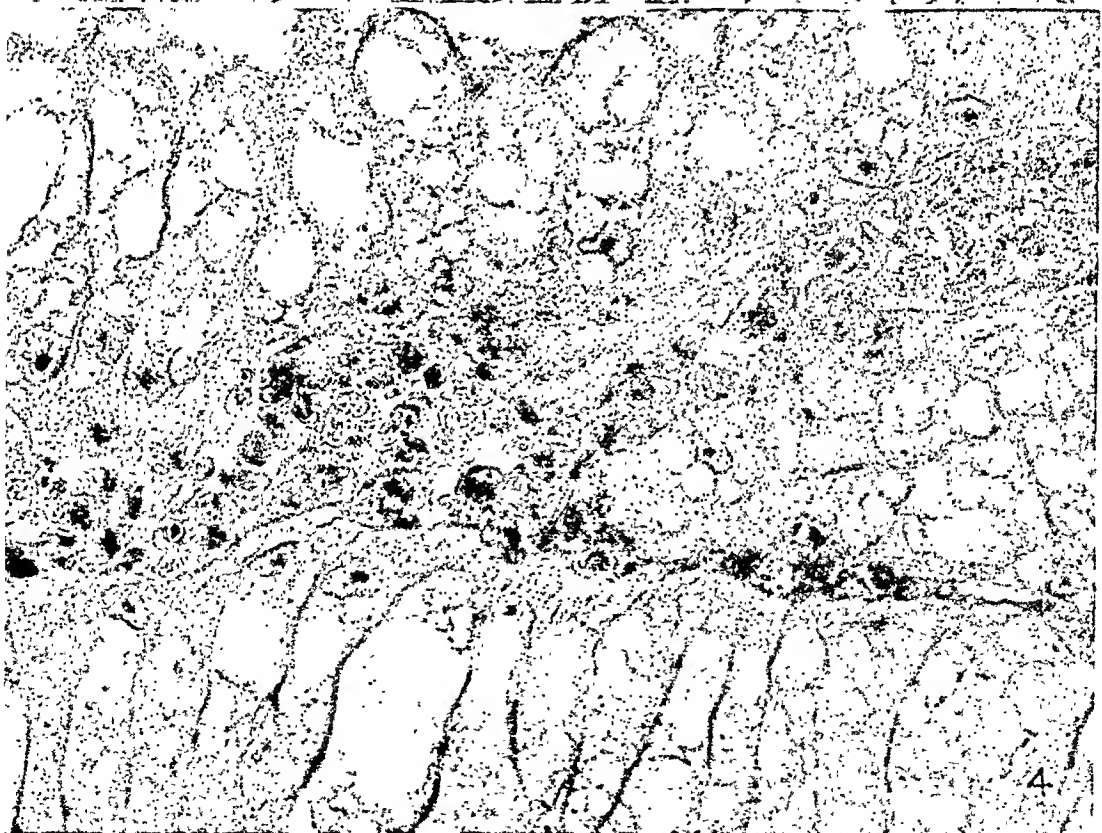
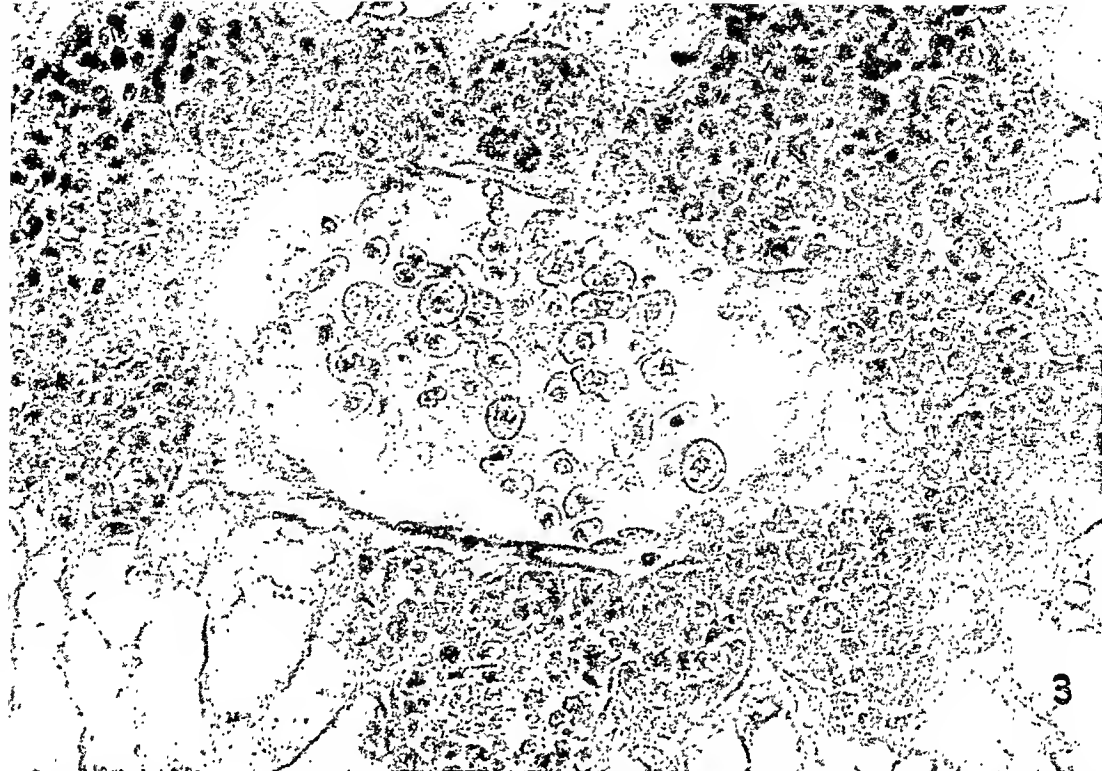


Fig. 3.—Yolk sac blood islet of an 11 day old chick embryo inoculated five days previously with 12.5 mg. of folic acid and four days previously with 0.01 mg. of 4-aminopteroylglutamic acid. There is diminution in the cellularity of the blood islet, but not as marked changes occurred as with the antagonist alone. $\times 333$.

Fig. 4.—Yolk sac blood islet of a 12 day old chick embryo previously inoculated on the sixth day of life with 3.75 U.S.P. units (injectable) of liver extract and on the seventh day with 0.01 mg. of 4-aminopteroylglutamic acid. There is marked diminution of the cellularity with loss of basophilia of the nuclei. $\times 333$.

of 5.5 Gm. of hemoglobin per hundred cubic centimeters of blood and 1,330,000 cells per cubic millimeter. The corresponding figures for the control series (series 2), inoculated only with distilled water, were 5.3 Gm. of hemoglobin and 1,340,000 cells. The blood islets of the chick embryos of series 1 and 2 showed no definite variation in comparison with the normal seven days after inoculation with N¹⁰-methylpterotic acid.

Series 3 and 4 consisted of embryonated eggs inoculated on the eighth day with 0.01 mg. of methyl-4-aminopteroylglutamic acid; those of series 4 also received 0.1 mg. of folic acid simultaneously. The yolk sac blood islets of embryos of both series showed some diminution of size and number but such alterations were much less than those occurring with comparable doses of 4-aminopteroylglutamic acid. The inoculation of 0.1 mg. amounts of folic acid seemed to diminish these changes. Only occasional fragmentation of the nuclear structure was observed.

COMMENT

Several folic acid inhibitors have been described.⁵ Martin and associates^{5a} were the first to describe a displacing agent (d(-)methyl folic acid) for pteroylglutamic acid, which they demonstrated by using *Streptococcus faecalis* (lactis) R assay technic. Seeger and associates^{5b} reported the synthesis of another folic acid analogue and antagonist, later referred to as 4-aminopteroylglutamic acid. By feeding a chemically unidentified folic acid antagonist to weanling female albino rats of the Wistar strain, Franklin and co-workers⁶ produced granulocytopenia, leukopenia and anemia within a period of three weeks. The bone marrow of a rat with a severe antagonist-induced syndrome showed an increase in the proportion of nucleated red blood cells. Deeply staining blast cells of the erythroid series were seldom seen in the controls but were numerous in the marrow of the animals receiving the antagonist. Few mature granulocytes were found in the marrow of the latter group of rats. Other characteristic signs of the deficiency syndrome were loss of weight, unkempt, ruffled fur, encrustation of the vibrissae with red pigment, severe diarrhea, ulcerations of the oral cavity and gingivitis. Administration of more than 0.3 mg. of pteroylglutamic acid per gram of antagonist prevented these changes. Administration of 100 mg. of pteroylglutamic acid per 10 Gm. of antagonist caused rapid recovery, with normal appearance, growth rates and blood picture within a few weeks. Following recovery, the animals showed unexplained splenomegaly. Asenjo⁷ has reported that splenic infarcts occur in rats on a diet deficient in folic acid.

5. (a) Martin, J. G.; Tolman, L., and Moss, J.: *Arch. Biochem.* **12**:318, 1947. (b) Seeger, D. R.; Smith, J. M., Jr., and Hultquist, M. E.: *J. Am. Chem. Soc.* **69**:2567, 1947. (c) Daniel, L. J.; Norris, L. C.; Scott, M. L., and Heuser, G. F.: *J. Biol. Chem.* **169**:689, 1947. (d) Wooley, D. W., and Pringle, A.: *ibid.* **174**:327, 1948.

6. Franklin, A. L.; Stokstad, E. L. R.; Belt, M., and Jukes, T. H.: *J. Biol. Chem.* **169**:427, 1947.

7. Asenjo, C. F.: *Federation Proc.* **7**:144, 1948.

Franklin and associates⁸ have also produced slow growth, anemia and leukopenia in both mice and chicks with a chemical antagonist of pteroylglutamic acid. Such signs were reversible. However, large amounts of 4-aminopteroylglutamic acid (1 milligram per kilogram of diet) produced death within a few days in mice, and this effect was not prevented by as much as 100 mg. of pteroylglutamic acid per kilogram of diet. Welch and co-workers⁹ have produced diminished growth rate, severe anemia, patchy alopecia and profuse diarrhea in a pig by feeding a purified diet, succinylsulfathiazole and a folic acid inhibitor. Reticulocytosis and rises of hemoglobin level and red cell count followed the changing of the diet to one containing the extrinsic factor.

Daniel and associates^{5c} have demonstrated that several synthetic pterins possess marked antibacterial activity which is antagonized competitively by folic acid. Such pterins have the unique capacity of inhibiting bacteria which synthesize folic acid as well as those requiring the preformed vitamin. Edwards and co-workers¹⁰ have described an experiment in which *Str. faecalis* utilization of folic acid was inhibited by a derivative of benzimidazole. Hutchings and co-workers¹¹ have reported pteroylaspartic acid to be an effective inhibitor of the utilizing of pteroylglutamic acid for growth by *Lactobacillus casei*, *Str. faecalis* R and the chick but not to be an effective inhibitor of such utilization by the rat. In the chick the inhibitor antagonized the growth effects of pteroylglutamic acid in a ratio of 500 to 1 by weight. Hall¹² has reported that quinoxaline can act as an inhibitor of the growth of *Streptococcus lactis* R and that pteroylglutamic acid can reverse the inhibition. Emerson and Mushett¹³ have observed that in rats maintained on a purified diet containing 1 per cent sulfathiazole and all identified vitamins except pteroylglutamic acid, granulocytopenia and leukopenia developed. Treatment with either purified liver extracts or pteroylglutamic acid resulted in prompt restoration of normal numbers of white cells. An increase of the number of reticulocytes that was considered significant was also reported. Swenseid and co-workers,¹⁴ during a study of a group of pteroylglutamic analogues and pterins, observed the development of leukopenia and/or anemia within a fourteen day period in weanling rats receiving purified diets containing succinylsulfathiazole. The pteroylglutamic acid analogues produced leukopenia and anemia, but the pterins had a preferential effect on the leukocytes in those particular experimental

8. Franklin, A. L.; Stokstad, E. L. R., and Jukes, T. H.: *Proc. Soc. Exper. Biol. & Med.* **65**:368, 1947.

9. Welch, A. D., and others: *Proc. Soc. Exper. Biol. & Med.* **65**:364, 1947.

10. Edwards, P. C.; Starling, D.; Mattocks, A. M., and Skipper, H. E.: *Science* **107**:119, 1948.

11. Hutchings, B. L., and others: *J. Biol. Chem.* **170**:323, 1947.

12. Hall, D. A.: *Biochem. J.* **41**:294, 1947.

13. Emerson, G. A., and Mushett, C. W.: *Federation Proc.* **7**:285, 1948.

14. Swenseid, M. E., and others: *Federation Proc.* **7**:299, 1948.

conditions. Minnich and Moore¹⁵ have produced normocytic anemia, leukopenia and thrombopenia, with hypoplastic marrows, in guinea pigs by daily intramuscular injection of 0.5 to 5.0 mg. of 4-aminopteroylglutamic acid. Neither liver extract nor folic acid, in the doses used, prevented the development of anemia, though folic acid did seem to prevent leukopenia and thrombopenia. Farber and associates¹⁶ have reported that remissions were induced in acute leukemia with folic acid antagonists, pteroylaspartic acid and 4-aminopteroylglutamic acid.

A new method of studying the effects of folic acid antagonists, folic acid, liver extract and vitamin B₁₂ is described. With this technic, 4-aminopteroylglutamic acid was found to be the most effective of the three antagonists used. The survival time of the inoculated embryos decreased as the dosage of the antagonist inoculated increased. With effective concentrations, detectable changes occurred in the level of hemoglobin and the total cell count. The cytologic changes in the blood islets of the yolk sac were marked. They were characterized by a decrease in both the number of the blood islets and their individual cellularity. The remaining nuclei showed pyknosis, karyolysis and karyorrhexis.

This effect, as well as that of the less active methyl-4-aminopteroylglutamic acid, appeared altered by injections of folic acid. However, liver injection U. S. P. and vitamin B₁₂, in the doses employed, did not alter the apparent effect of the 4-aminopteroylglutamic acid. N¹⁰-methylpteroic acid in relatively larger doses than the other antagonists caused no detectable changes in the hemoglobin level or the cell count of the blood. The blood islets of the yolk sacs of the embryonated eggs inoculated with it appeared essentially normal seven days after the injection.

SUMMARY

A new technic has been employed for the study of folic acid inhibitors. By this technic it was shown that 4-aminopteroylglutamic acid caused marked changes in the hemopoietic tissue of embryonated eggs comparable to those described in other species. The blood islets of the yolk sac showed diminution in number and size with decreased cellularity. The remaining nuclei showed pyknosis, karyolysis and karyorrhexis. This effect was less marked in the presence of relatively large amounts of folic acid. In the dose employed, liver injection U. S. P. (solution liver extract purified,[®] 15 U. S. P. units per cubic centimeter) and vitamin B₁₂ did not alter the effects of 4-aminopteroylglutamic acid in the yolk sac blood islets. N¹⁰-methylpteroic acid in relatively large doses had no appreciable effect. Methyl-4-aminopteroylglutamic acid caused much less change in the blood islets than did comparable doses of 4-aminopteroylglutamic acid.

15. Minnich, V., and Moore, C. V.: *Federation Proc.* **7**:276, 1948.

16. Farber, S., and others: *New England J. Med.* **238**:787, 1948.

CHOLESTEROL OF THE HUMAN ADRENAL GLAND

Its Significance in Relation to Adrenal Function and Structure

WALTER F. ROGERS Jr., M.D.

SYRACUSE, N. Y.

AND

ROBERT H. WILLIAMS, M.D.

SEATTLE

PREVIOUS investigations of the cholesterol of the adrenal gland¹ have led authors to conclude that, in general, the adrenal cholesterol levels were low in persons who died of severe infections, moderately low in chronic debilitating and malignant diseases and high in people who died of cardiovascular and/or renal disorders.

Recent progress in knowledge of the function of the adrenal gland has revealed certain facts, listed below, that facilitate the interpretation of the previous findings and prompt a reevaluation of the adrenal cholesterol in regard to its functional significance and its level in normal and in diseased states.

1. Animals that are subjected to nonspecific types of acute stress have a rapid fall of the adrenal cholesterol, provided the hypophysis of the experimental animal is intact.²

2. An extract of the anterior lobe of the pituitary gland containing the adrenocorticotrophic principle when administered to animals causes a decrease in the cholesterol of the adrenal gland accompanied by changes similar to those that occur when adrenal cortex extracts are given.³

From the Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard), Boston City Hospital, and the Department of Medicine, Harvard Medical School, Boston.

1. (a) Chauffard, A.; Laroche, G., and Grigaut, A.: *Compt. rend. Soc. de biol.* **73**:23, 1912; (b) **76**:529, 1914. (c) Fex, J.: *Biochem. Ztschr.* **104**:82, 1920. (d) Kohno, T.: *Folia endocrinol. japon.* **4**:60, 1928; (e) **4**:85, 1929.

2. Sayers, G.; Sayers, M. A.; Fry, E. G.; White, A., and Long, C. N. H.: *Yale J. Biol. & Med.* **16**:361, 1944. Long, C. N. H.: *Recent Progress in Hormone Research*, New York Academic Press, Inc., 1947, vol. 1, pp. 99-117.

3. Sayers, G.; Sayers, M. A.; White, A., and Long, C. N. H.: *Proc. Soc. Exper. Biol. & Med.* **52**:200, 1943. Sayers, G.; Sayers, M. A.; Liang, T., and Long, C. N. H.: *Endocrinology* **38**:1, 1946. Jensen, H., and Gratton, J. F.: *Am. J. Physiol.* **128**:270, 1940. Tyslowitz, R., and Astwood, E. B.: *ibid.* **136**:22, 1942. White, A., and Dougherty, T. F.: *Ann. New York Acad. Sc.* **46**:859, 1946.

3. In man the administration of the anterior pituitary extract containing the adrenocorticotrophic factor causes an increased excretion of "cortins" and 17-ketosteroids.⁴

Two important features of adrenal cholesterol are apparent; it fluctuates widely and rapidly, depending on the exogenous and endogenous stimuli to which the organism has been subjected, and it appears to play an important role in the biochemical reactions of the adrenal cortex.

Therefore, we have attempted to define the range of the normal human adrenal cholesterol with emphasis on the fact that adrenal glands may be regarded as normal only when they are obtained from healthy persons who have died suddenly and who at autopsy show no pathologic changes other than those caused by the fatal agent. We have also determined the cholesterol content of adrenal glands of persons suffering from cardiac, renal, infectious, hepatic, cancerous and endocrine diseases and have correlated the findings with published data on the urinary excretion of "cortin-like" compounds⁵ and 17-ketosteroids.⁶ In correlating adrenal cholesterol levels with disease states, greater significance is attached to the findings after sudden death, since nonspecific changes in the adrenal gland from chronic illness, infections and therapeutic measures are at a minimum.

Tissue sections of the same adrenal glands were examined, and the histologic changes accompanying decreased and increased amounts of cholesterol are described. The latter group includes such lesions as "focal and nodular hyperplasia" and small adrenal adenomas. The functional significance and the genesis of the lesions associated with increased amounts of cholesterol are postulated on the basis of (1) the adrenal response to stress, (2) a comparison with the hyperplasia that occurred in 4 proved cases of hyperadrenocorticism and (3) the experimental data concerning the depletion and the accumulation of cholesterol in adrenal cortices.

MATERIALS AND METHODS

The materials and the methods used in this study were similar to those of a previous study.⁷ Special histochemical methods, such as staining with phenyl-

4. Mason, H. L.; Power, M. H.; Rynearson, E. H.; Ciaramelli, L. C.; Li, C. H., and Evans, H. M.: *J. Biol. Chem.* **169**:223, 1947. Forsham, P. H.; Thorn, G. W.; Prunty, F. T. G., and Hills, A. G.: *J. Clin. Endocrinol.* **8**:15, 1948.

5. (a) Talbot, N. B.; Albright, F.; Saltzman, A.; Zygmuntowicz, A., and Wixom, R.: *J. Clin. Endocrinol.* **7**:331, 1947. (b) Venning, E. H., and Browne, J. S. L.: *ibid.* **7**:79, 1947. (c) Daughaday, W. H.; Jaffe, H., and Williams, R. H.: *ibid.* **8**:66, 1948. (d) Shipley, R. A.; Dorfman, R. I.; Buchwald, E., and Ross, E.: *J. Clin. Investigation* **25**:673, 1946.

6. Salter, W. T.; Cahen, R. L., and Sappington, T. S.: *J. Clin. Endocrinol.* **6**:52, 1946. Bruger, M.; Rosenkrantz, J. A., and Lowenstein, B. E.: *Am. J. M. Sc.* **208**:212, 1944. Venning and Brown.^{5b}

7. Rogers, W. F., Jr., and Williams, R. H.: *Arch. Path.* **44**:126, 1947.

hydrazine and examining by means of ultraviolet rays, were not used because of the previously reported nonspecificity of these methods.⁷

ADRENAL CHOLESTEROL

The adrenal cholesterol values of the 11 cases that fulfilled the criteria of normality are given in table 1. They relate to 7 males and 4 females, ranging in age from 18 to 62, with an average age of 34.6 years. The normal cholesterol concentration ranged from 5.35 to 8.20 Gm. per hundred grams of tissue, and the total cholesterol content from 485 to 710 mg. There was no apparent correlation between age and adrenal weight, cholesterol concentration or total cholesterol content. The combined weight of the adrenal glands was greater in males than in females, but the cholesterol concentration tended to be higher in females, resulting in a fairly equal total cholesterol content.

The cholesterol concentration and content of adrenal glands obtained in cases of various types of cardiovascular and/or renal disease and Cushing's syndrome

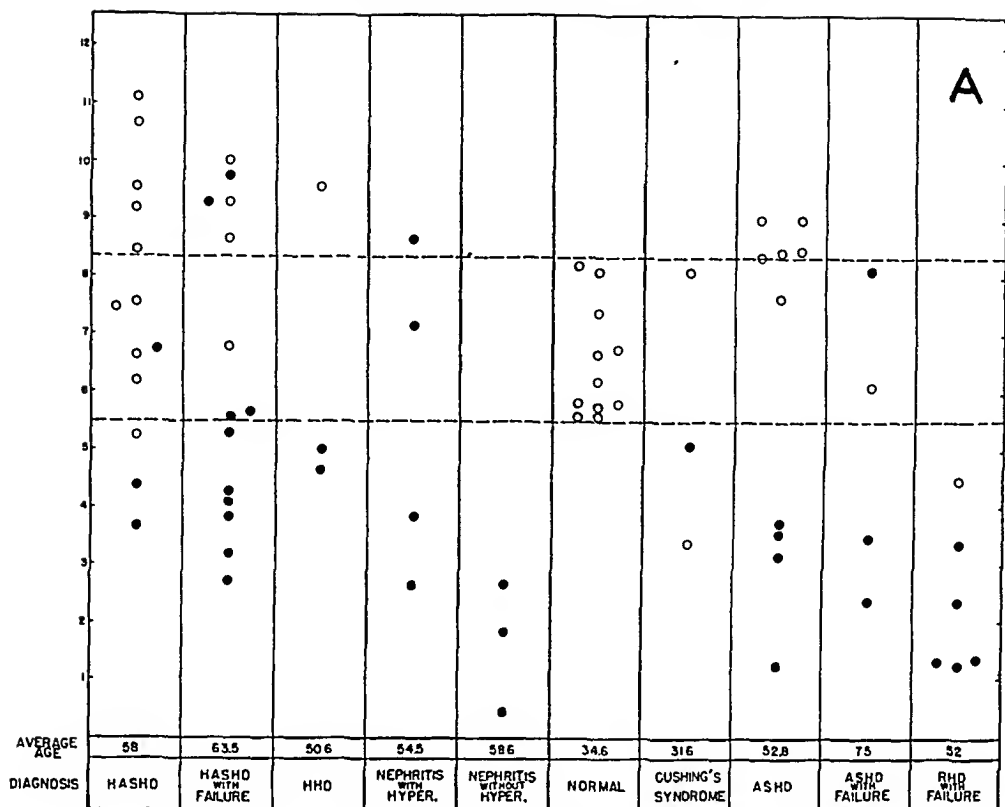
TABLE 1.—*Normal Persons Who Suffered Sudden Death*

Patient	Age, Yr.	Combined Adrenal Weight, Gm.	Cholesterol Concentration, Gm. per 100 Gm. of Tissue	Total Cholesterol, Mg.
Males				
H. W.....	18	10.54	5.35	564
W. D.....	24	9.44	7.28	637
E. S.....	25	8.78	6.20	544
J. T.....	26	9.18	5.75	528
J. W.....	38	8.98	5.58	501
H. G.....	57	9.16	6.81	624
C. M.....	62	9.45	5.55	524
Average.....	35.7	9.37	6.07	567
Females				
A. R.....	24	8.20	6.66	546
M. R.....	30	8.00	8.02	642
H. C.....	35	8.64	5.62	485
M. F.....	42	8.78	8.20	720
Average.....	32.7	8.40	7.12	593

are shown in figure 1. The cases are further divided into those in which death was sudden and those in which death was prolonged. In all cases in which death was considered as sudden, the survival time was a matter of minutes, the longest interval being forty-five minutes.

It is evident from figure 1 that in patients with hypertensive arteriosclerotic heart disease, with or without congestive heart failure, a significant number had cholesterol concentrations and total cholesterol contents which were higher than normal. Abnormal values were also found in hypertensive heart disease in regard to the total cholesterol content. In cases of arteriosclerotic heart disease without failure sudden death was associated with slightly high values, whereas low values were found after prolonged illness. Patients with rheumatic heart disease and congestive heart failure had low cholesterol values. Cases of nephritis are divided into those with and those without hypertension. Nephritic patients with hypertension had higher levels of cholesterol. Regardless of the disease entity, patients who died suddenly had higher adrenal cholesterol concentrations and total cholesterol contents than those who suffered a prolonged death. The influence of age on adrenal cholesterol did not appear to be significant, as it is noted (fig. 1) that

ADRENAL CHOLESTEROL CONCENTRATION



TOTAL ADRENAL CHOLESTEROL IN MGS.

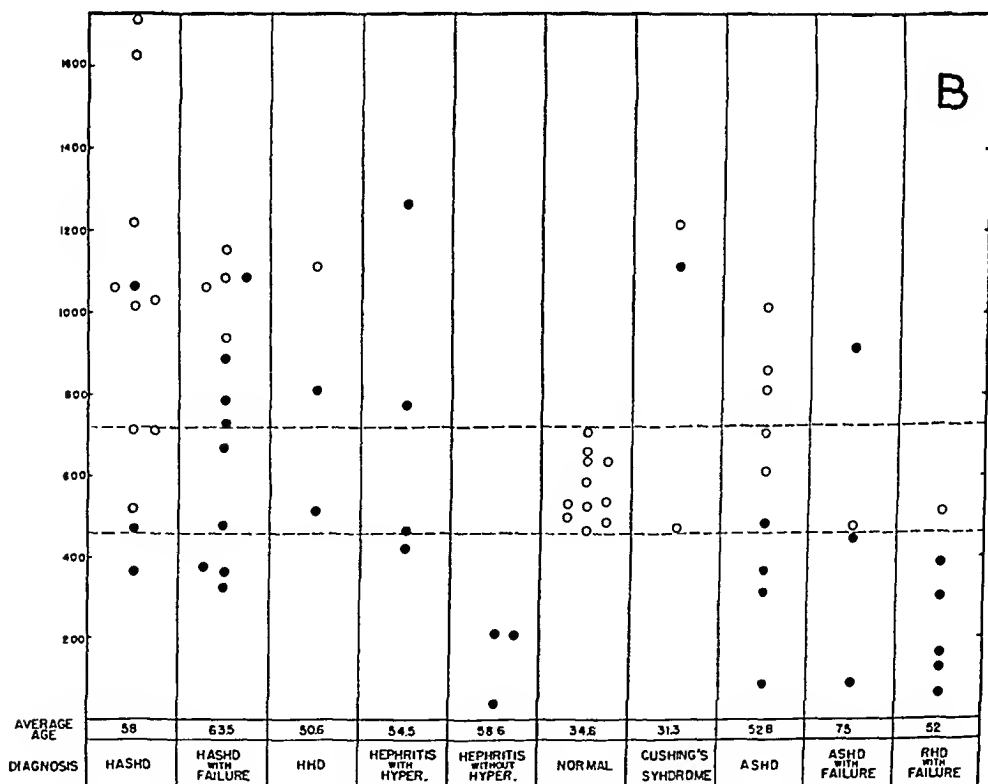


Fig. 1.—Cholesterol of human adrenal glands. *A*, cholesterol concentration in grams per hundred grams of tissue. Open circles denote persons who died suddenly; solid circles, those whose deaths were prolonged. *HASHD* signifies hypertensive arteriosclerotic heart disease; *HHD*, hypertensive heart disease; *Hyper.*, hypertension; *ASHD*, arteriosclerotic heart disease; *RHD*, rheumatic heart disease.

B, total adrenal cholesterol content in milligrams. The symbols and abbreviations are explained in the legend for *A*.

most groups had comparable average ages and yet distinct group variations in respect to adrenal cholesterol existed.

In the group of patients with endocrinopathies, table 2, there were 3 patients who had Cushing's syndrome. Each of the patients had classic symptoms and signs of this disorder, with confirmatory laboratory data. One patient, E. M., a 20 year old unmarried woman, had had symptoms of the disease for one year. Exploration of the adrenal glands revealed bilateral diffuse hyperplasia, and partial adrenalectomy was performed. The portion of the adrenal glands removed had a cholesterol concentration of 3.32 Gm. per hundred grams of adrenal tissue. The total weight of both adrenal glands was postulated to be at least 14 Gm. because of the fact that approximately 90 per cent of one adrenal gland was removed, the weight of this portion being 7 Gm., and both adrenal glands appeared to be of equal size at operation. A 38 year old man who had suffered from Cushing's disease for three and a half years died of acute intestinal obstruction of two days'

TABLE 2.—*Weight of Adrenal Glands and Cholesterol Levels of Patients Who Had Endocrinopathies*

Patient	Diagnosis	Age, Yr.	Sex	Combined Adrenal Weight, Gm.	Cholesterol Concentration, Gm. per 100 Gm. of Tissue	Total Cholesterol, Mg.
E. M.	Cushing's disease	20	F	14.00*	3.32	465
A. G.	Cushing's disease	38	M	22.01	5.08	1,118
H. L.	Cushing's disease	36	F	15.02	8.03	1,206
B. M.	Adrenogenital syndrome	12	F	†	1.92	...
M. J.	Addison's disease	42	F	30.00‡	0.62	186
R. M.	Anorexia nervosa	18	M	14.21	0.25	35
R. B.	Thyrotoxicosis	55	F	10.54	0.43	45
A. L.	Thyrotoxicosis	57	M	10.02	4.03	403
L. L.	Myxedema	59	F	9.45	1.50	142

* See text for derivation of this figure.

† The analysis was made from 90 per cent of the left adrenal gland which was removed at operation.

‡ This patient had Addison's disease on the basis of amyloidosis, which explains the abnormally heavy weight of the adrenal glands.

duration. At autopsy, the adrenal glands were bilaterally enlarged and weighed 22.01 Gm. The cholesterol concentration was 5.08 Gm. per hundred grams of tissue, resulting in a total cholesterol content of 1,118 mg. The third patient was a 36 year old woman who had had signs and symptoms of Cushing's disease for about five years. While in the hospital she suddenly became irrational and comatose and died of a pontile hemorrhage. Her adrenal glands were bilaterally enlarged, the combined weight being 15.02 Gm. The total cholesterol content was 1,206 mg., and the cholesterol concentration was 8.03 Gm. per hundred grams of tissue.

Another patient with hyperadrenocorticism (a 12 year old girl with the adrenogenital syndrome) had 90 per cent of her left adrenal gland removed at operation. The cholesterol concentration of this tissue was 1.92 Gm. per hundred grams of tissue. Previous laparotomy had established the fact that both glands were the site of hyperplasia and that no ovarian or adrenal tumor was present.

One patient with Addison's disease, caused by amyloidosis, had low adrenal cholesterol values. In a case in which an 18 year old sailor had anorexia nervosa, the adrenal cholesterol value was markedly diminished despite the increased

size of the adrenal glands. In 2 cases of thyrotoxicosis the cholesterol values were moderately depressed in one and markedly so in the other.

There were 8 cases of diabetes mellitus in this series. However, all cases were complicated by acute and chronic infections or heart disease, so that the values obtained were not suitable for analysis.

The adrenal cholesterol values of patients with infections were low. In acute infections the average cholesterol concentration was 1.97 mg. per hundred grams of tissue and the average total cholesterol content was 266 mg., the lowest values being noted in cases of meningococcic meningitis and pneumococcic pneumonia. In chronic infections the average cholesterol concentration was 4.44 Gm. per hundred grams of tissue with an average total cholesterol content of 447 mg.

In patients with cancers both the cholesterol concentration and the total cholesterol content were low, averaging 2.09 Gm. per hundred grams of tissue and 311 mg., respectively.

TABLE 3.—*Weight of Adrenal and Cholesterol Levels of Patients Who Suffered from Miscellaneous Diseases*

Diagnosis	Age	Sex	Tempo of Death	Combined Adrenal Weight, Gm.	Cholesterol Concentration, Gm. per 100 Gm. of Tissue	Total Cholesterol, Mg.
Periarteritis nodosa.....	59	F	Prolonged	9.69	3.83	371
Disseminated lupus erythematosus	26	F	Prolonged	11.79	0.28	33
Alcoholic cirrhosis.....	52	M	Prolonged	12.05	6.27	755
Alcoholism *.....	29	F	Sudden	11.10	1.84	204
Alcoholism.....	31	M	?	10.41	6.33	659
Methyl alcoholism poisoning.....	30	F	Prolonged	8.47	5.05	427
Avitaminosis.....	22	F	Prolonged	9.60	3.13	300
Hemochromatosis.....	41	M	Prolonged	9.40	0.82	77
Hemochromatosis.....	47	M	Prolonged	1.74	...
Pregnancy †.....	47	F	Sudden	14.53	4.63	673
Duodenal ulcer ‡.....	60	M	Sudden	11.55	7.45	860

* The patient died of a fractured skull.

† The patient's death was due to an anesthetic accident; abscess of a lung was found at necropsy.

‡ The patient died of pulmonary embolism.

In table 3 pertinent data are given concerning a variety of diseases which were observed. The diverse nature of the cases makes analysis of the results impracticable.

HISTOLOGIC OBSERVATIONS

The normal adrenal cortex contains three distinct zones, namely, the zonae glomerulosa, fasciculata and reticularis. The cells of the normal zona glomerulosa and zona fasciculata have a fluffy and vacuolated appearance, while the cells of the zona reticularis are more acidophilic and frequently contain a yellow-brown pigment. Individual cells (fig. 2A) of the zonae glomerulosa and fasciculata have round nuclei that are fairly light staining, with a moderate number of evenly distributed dark granules. Radiating from the nuclei to somewhat ill defined cell membranes are fine lacelike strands of acidophilic material that enclose small vacuolated spaces, which contain lipid substances.

Whenever the body is subjected to noxious agents, a marked alteration occurs in the cells of the zona glomerulosa and the zona fasciculata (fig. 2B). The

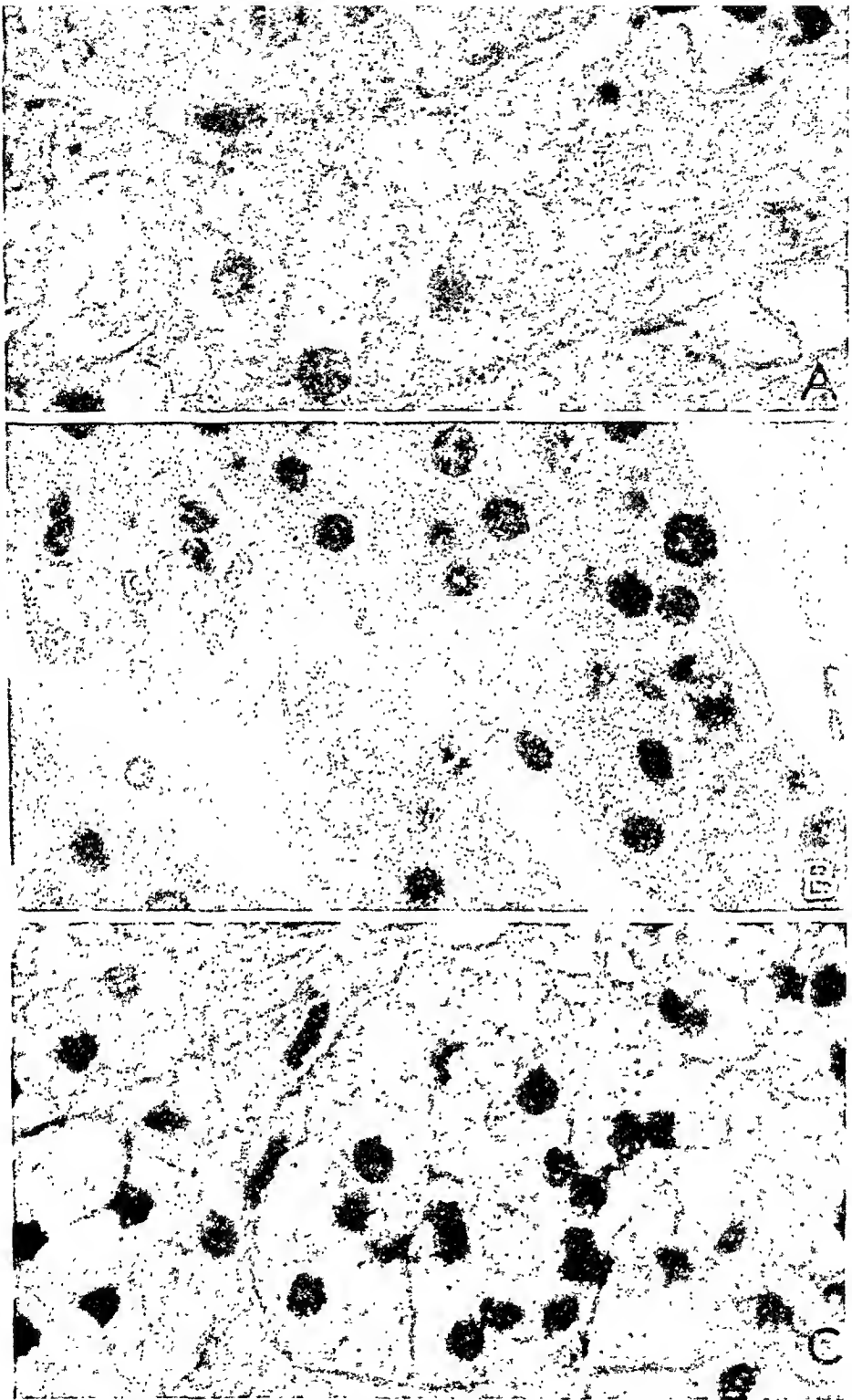


Fig. 2.—*A*, high power view of an outer region of the zona fasciculata of the cortex of a normal adrenal gland. Phloxine-methylene blue; $\times 1,353$.

B, area of the zona fasciculata of the cortex of an adrenal gland from a person who died of an acute hemolytic streptococcus infection of three days' duration. Note the absence of vacuoles and the density of the cytoplasm as compared with the normal zona fasciculata in *A*. Phloxine-methylene blue; $\times 1,353$.

C, histologic change accompanying increase of adrenal cholesterol: type 1. Compare with *A* and note that cell size has increased, vacuoles are larger and nuclei are smaller but that the lacelike cytoplasmic structure has been maintained. Zona fasciculata; phloxine-methylene blue; $\times 1,353$.

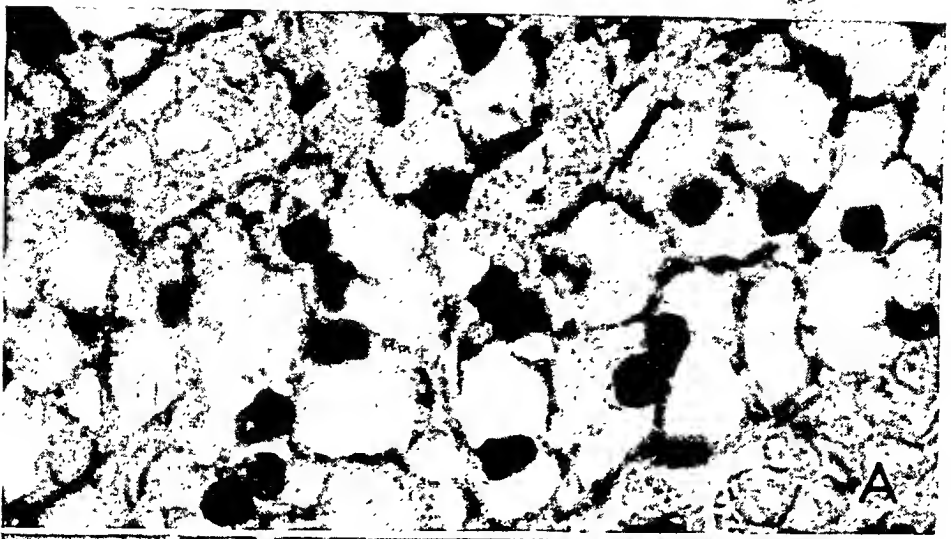


Figure 3
(See legends on opposite page)

vacuoles of these cells disappear, and the cytoplasm becomes diffusely acidophilic. These histologic changes characteristic of lipid depletion are accompanied by a fall of cholesterol and are quantitatively well correlated with the latter.

Adrenal glands that have abnormally high levels of cholesterol also have characteristic histologic appearances that reflect the change of lipid content. We have divided these morphologic alterations into three essential types.

Type 1 is a slight alteration of the normal appearance (fig. 2 *C*), in which the cells are somewhat larger in circumference and the vacuoles appear larger but the cytoplasm retains its delicate framework of acidophilic strands. The nuclei of these cells tend to be smaller and more pyknotic than those of normal cells, and the cell membranes are less distinct.

Type 2 (fig. 3 *A*) is characterized by cells that have small hyperchromatic nuclei that are frequently forced close together by large, round, clear vacuolated spaces filled with lipid substances. In contrast to type 1 the delicate strands of acidophilic framework are sparse, do not stretch across the large clear spaces and are compressed to form the boundaries of these vacuoles.

Type 3 is characterized by small conglomerations of adrenal cortical cells, each group being separated from the others by distinct, moderately thick strands of acidophilic material (fig. 3 *B*). Individual conglomerations contain a varying number (usually 5 to 10) of hyperchromatic, frequently distorted nuclei. Single cell membranes may or may not be evident, but lacelike acidophilic cytoplasmic strands are prominent, producing a fine spongy, vacuolated, appearance. The change in cell structure described as type 3 is the same as has been described in focal and nodular hyperplasia and small adrenal adenomas.

Although three distinct histologic appearances are described, one, two or all three types of change may be observed in a single adrenal cortex, accompanied by transitions between types. In cases of cardiovascular and/or renal disease all three types of alteration were frequently noted (56 per cent of the cases) and when present in a moderate or marked degree adequately accounted for the increased cholesterol values. However, these changes are not specific, for they were found, to a minimal degree, in 18 per cent of the patients dying of other causes.

The degree to which abnormal adrenocortical cells respond to alarming stimuli is of considerable interest, particularly in patients with cardiovascular and/or renal disease. In figure 1 it is apparent that patients of this type whose death was prolonged had lower adrenal cholesterol values than those whose death was sudden, suggesting that alarm produced an adrenal response similar to normal. However, the histologic appearance indicates a response which differs from normal in that the cells which were responsible for the abnormally high levels of cholesterol did not show evidence of depletion of lipid and development of diffusely acidophilic cytoplasm. The accompanying photomicrographs serve to illustrate this point.

The response of type 1 cells is represented in the adrenal glands from a 21 year old woman who died of uremia and chronic pyelonephritis. She had moderate

Fig. 3.—Histologic change accompanying increase of adrenal cholesterol: *A*, type 2. Compare with figure 2 *A* and note the small, hyperchromatic nuclei and the large, completely vacuolated spaces. Zona fasciculata; phloxine-methylene blue; $\times 1,211.5$.

B, type 3. Note the small conglomerations of cells separated by thick strands of acidophilic staining material. The cytoplasm retains a lacelike appearance. Phloxine-methylene blue; $\times 1,211.5$.

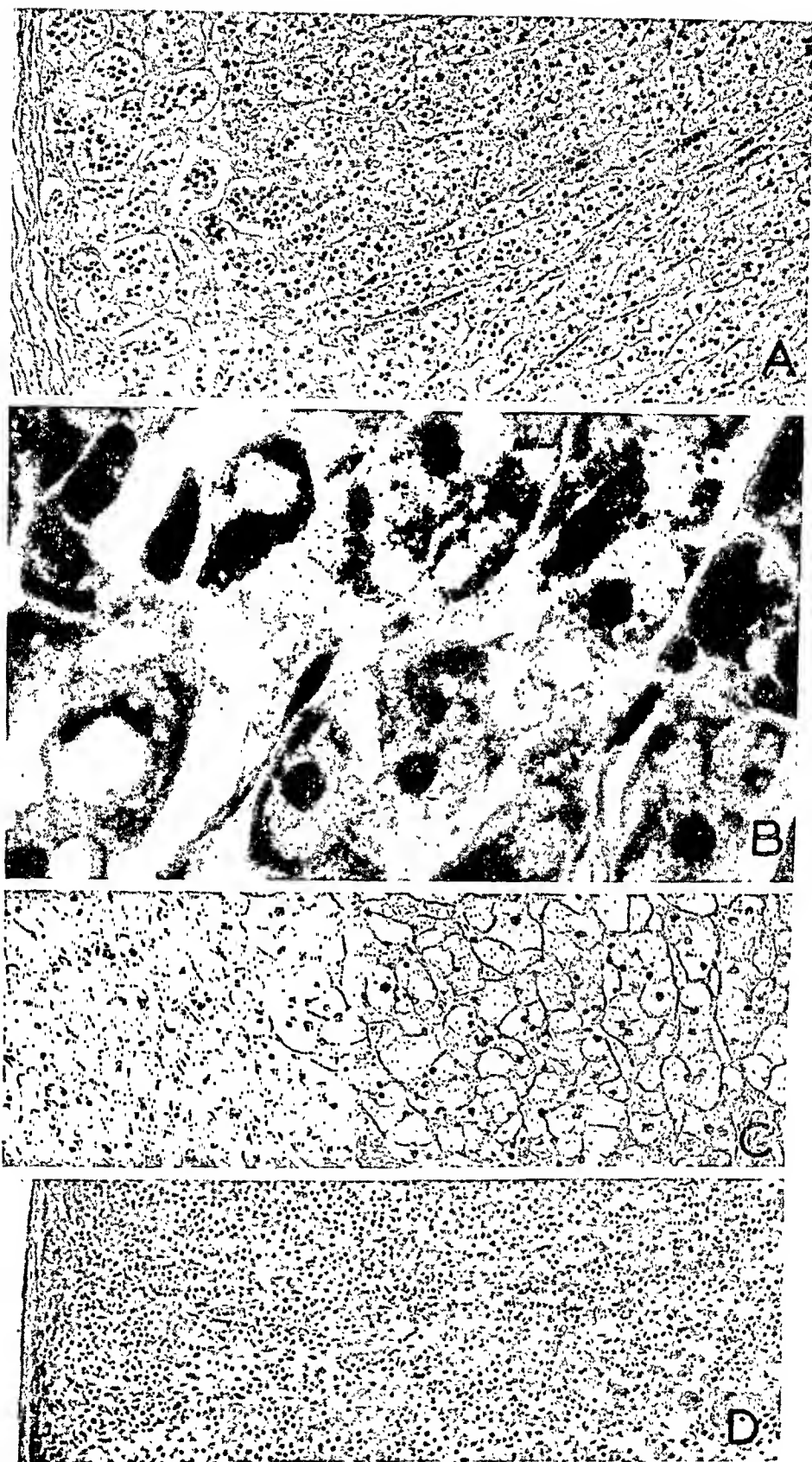


Figure 4

(See legends on opposite page)

hypertension and marked anasarca. During her last eight days of life she had severe potassium intoxication. At autopsy her adrenal glands weighed 14.97 Gm. The adrenal cholesterol concentration was 8.51 Gm. per hundred grams of tissue, and the total cholesterol content was 1,274 mg. Histologic examination revealed that the adrenal cortex was composed almost completely of cells that have been described as those of type 1 (fig. 4*A*). Only an occasional cell showed evidence of having responded to stress, the rest being completely filled with lipid, despite her severe illness.

Figure 4*B* shows a section of an adrenal gland from a 60 year old man who had hypertension and an acute myocardial infarction. He lived for four days after the onset of his symptoms of coronary occlusion, during which time he was severely ill, as manifested by shock, cyanosis, dyspnea and other signs of heart failure. Although the cells of the adrenal cortex showed signs of depletion, large clear vacuoles were still evident and apparently had not taken part in the gland's discharge of lipid substances.

Finally, in focal and nodular hyperplasia and in adrenal adenomas, evidence of lipid depletion is most always lacking, although in a few cases a small number of depleted cells were sometimes seen. An example of this lack of response is shown in figure 4*C*, which illustrates a section of an adrenal gland from a 56 year old man who died of arteriosclerotic heart disease and congestive heart failure. There is no evidence of depletion of lipid in the area of focal hyperplasia (type 3 cells), while in the area of normal structure the cells show diffusely acidophilic cytoplasm.

The presence of the lesions just described, some of which have been called "adrenal hyperplasia," results in an adrenal gland that has a varied histologic pattern from one area to another. In contrast was the adrenal hyperplasia noted in cases of clinical hyperadrenocorticism that consisted of regular and marked cellular proliferation (fig. 4*D*). Not only was the structure uniform, but the absence of cells characteristic of a high lipid content was notable. This would be expected in patients E. M. and B. M. (table 2), both of whom had low cholesterol values. In patients H. L. and A. G., who had higher cholesterol values, cells of types 1 and 2 were seen, but their extent was minimal and overshadowed by the predominant abnormality of diffuse hyperplasia consisting of cells of normal or low lipid content.

COMMENT

There are few figures in the literature with which to compare our normal values of adrenal cholesterol concentration (5.35 to 8.20 Gm. per hundred grams of tissue) and total adrenal cholesterol content (485 to

Fig. 4.—*A*, adrenal cortex from a 21 year old woman with uremia and potassium intoxication. Despite her severe illness, note the apparent lack of adrenal response as evidenced by lipid-filled cells. Both adrenal cortices had this appearance throughout. Phloxine-methylene blue; $\times 184$.

B, zona fasciculata of an adrenal cortex showing evidence of depletion. However, the large clear vacuoles remain and show no evidence of depletion. Compare with figure 3*A*. Phloxine-methylene blue; $\times 1,289$.

C, section of an adrenal cortex from a person who died of arteriosclerotic heart disease and congestive heart failure. Note the depletion of cells in an area of normal structure at the left, but the absence of such a change in an area of focal hyperplasia at the right. Phloxine-methylene blue; $\times 230$.

D, adrenal cortex of E. M., table 3, with Cushing's syndrome. Note the regular and extensive hyperplasia and the absence of cells with a high lipid content. Phloxine-methylene blue; $\times 115$.

720 mg.). In 1935 Ewert⁸ published values of the adrenal cholesterol of 11 persons whom he considered to be normal. Six cases were collected from the literature, and several criticisms may be made. For instance, 2 of these cases were taken from Wachter and Hueck⁹; one person was killed in a railroad accident, while the other died of lysol[®] poisoning. The adrenal cholesterol concentration in these persons was 1.6 and 2.4 Gm. per hundred grams of tissue, respectively. However, the authors did not say whether death was sudden or not, or whether the rest of the organs were without evidence of disease at autopsy. Ewert quoted Fex,¹⁰ who reported "normal" adrenal cholesterol values of 4 persons. Of these 4 persons, only 1 may be considered normal, a 30 year old woman who died suddenly of suffocation and whose adrenal cholesterol concentration was 4.0 Gm. per hundred grams of tissue. The remaining patients died from one to four days after their fatal accident, which was adequate time for an "alarm reaction" to take effect on the adrenal cholesterol. Ewert's own normal subjects appear to have been persons who died suddenly. He reports values ranging from 2.8 to 5.1 Gm., with an average of 3.9 Gm., per hundred grams of tissue. He used a gravimetric procedure to determine the cholesterol content and commented that such a procedure gives values approximately 30 per cent lower than colorimetric methods.

Low cholesterol values are found in the adrenal glands of persons dying of such conditions as acute infections, burns and trauma. Living persons subjected to these same types of stress have shown, by both biologic and chemical methods, increased excretion of "cortin-like" compounds¹⁰ and of 17-ketosteroids—the latter transiently rising for from one to three days, followed by a period in which the excretion is usually below normal.¹¹

In chronic diseases the adrenal cholesterol values are moderately depressed. There are too few estimations of the "cortin" excreted in comparable living persons to draw conclusions, but from isolated reports it appears that the levels may be low, normal or increased. However, the amount of 17-ketosteroids excreted by chronically ill and debilitated persons is usually below normal.¹¹ Talbot and co-workers^{5a} and Venning and Browne^{5b} pointed out that glycogenic corticoid compounds

8. Ewert, B.: *Upsala läkaref. förh.* **40**:421, 1935.

9. Wachter, L., and Hueck, W.: *Arch. f. exper. Path. u. Pharmacol.* **71**:373, 1913.

10. Weil, P., and Browne, J. S. L.: *J. Clin. Investigation* **19**:772, 1940. Shipley, R. A.; Dorfman, R. I., and Howitt, B. N.: *Am. J. Physiol.* **139**:742, 1943. Venning, E. H.; Hoffman, M. M., and Browne, J. S. L.: *J. Biol. Chem.* **148**:455, 1943. Talbot, Albright, Saltzman, Zygmuntowicz and Wixom.^{5a} Venning and Browne.^{5b} Shipley, Dorfman, Buchwald and Ross.^{5d}

11. Forbes, A. P.; Donaldson, E. C.; Reifenstein, E. C., Jr., and Albright, F.: *J. Clin. Endocrinol.* **7**:264, 1947.

and 17-ketosteroids frequently do not parallel each other. When glyco-genic corticoid compounds increase, the excretion of 17-ketosteroids tends to be depressed. Selye¹² attributed this phenomenon to a "hormonal shift" in which during times of stress the adrenal gland increases its production of corticoid compounds but decreases its production of testoid compounds.

Thus, the evidence so far accumulated strongly points to the fact that under stress, particularly that of an acute nature, the cholesterol content of the human adrenal gland decreases and that this change is accompanied by an increased excretion of "cortins."

On the other hand, it cannot be said that whenever the adrenal cholesterol is low the excretion of "cortins" is high. This is borne out by the fact that in Addison's disease, panhypopituitarism, anorexia nervosa and certain chronic debilitating diseases in their terminal states, the adrenal cholesterol concentration is low, as is the excretion of "cortins."

Cases of hyperadrenocorticism afford the best examples for study of adrenal cholesterol where adrenal hyperfunction is of a sustained nature. We did not find the adrenal cholesterol concentration above normal in any case. It was very low in the patient with the adreno-genital syndrome and moderately depressed in 1 patient with Cushing's syndrome. Although the adrenal glands of both these patients were removed at operation, and some "alarming" stimuli occurred, it seems likely that the glands were extirpated before the adrenal cholesterol values were affected significantly. The elevation of the total adrenal cholesterol contents of the remaining 2 patients with Cushing's syndrome is attributable to the increased weight of the adrenal glands, not to an abnormally high adrenal cholesterol concentration.

As a decreased concentration of adrenal cholesterol may be associated with an increased or a decreased production of "cortins," and a high concentration of adrenal cholesterol was not present in cases of hyperadrenocorticism, it seems apparent that the rate of hormone production is not dependent on the concentration of adrenal cholesterol.

The clinical observations that Cushing's syndrome is usually associated with hypertension, and Addison's disease with hypotension, and that desoxycorticosterone may produce hypertension in patients with Addison's disease¹³ and an increase of blood pressure in normal persons¹⁴ arouse interest in the relation of function of the adrenal gland to hyper-

12. Selye, H.: *J. Clin. Endocrinol.* **6**:117, 1946.

13. Thorn, G. W.; Dorrance, S. S., and Day, E.: *Ann. Int. Med.* **16**:1053, 1942. Ferrebee, J. W.; Ragan, C.; Atchley, D. W., and Loeb, R. F.: *J. A. M. A.* **113**:1725, 1939.

14. Perera, G. A.; Knowlton, A. I.; Lowell, A., and Loeb, R. F.: *J. A. M. A.* **125**:1030, 1944.

tension. This interest is furthered by the results of animal experiments. Selye¹² has produced vascular lesions similar to those seen in hypertension and nephrosclerosis by the administration of desoxycorticosterone and crude anterior pituitary lobe extracts. Investigators¹⁵ have found that adrenalectomy reduces an animal's response to renin and that the adrenal cortex is necessary to maintain hypertension in Goldblatt animals. Thus, the finding of increased amounts of cholesterol in some adrenal glands from people who die of cardiovascular and/or renal disease, coupled with the fact that there is a decrease of adrenal cholesterol associated with elaboration of adrenal cortical hormones, poses the problem of the significance of the elevated adrenal cholesterol values in relation to hypertension.

Do these high cholesterol values mean continual increased production of adrenal hormones, or potentially increased production of hormones, or are they merely the reflection of an adrenal gland which has undergone repeated cycles of stimulation, depletion and "involution"? We believe that the last explanation is the most likely, although it is impossible to exclude the first two possibilities.

In regard to continual increased production of hormones it seems probable that if the function of adrenal cortex is an etiologic factor of hypertension, the electrolyte-controlling hormones of the desoxycorticosterone type play the most important role. Unfortunately, there is at present no satisfactory method of assaying the excretion of the hormones or of their products which influence electrolyte metabolism. Thus, important as they are, determinations of the output of this type of adrenal hormone in normal and in hypertensive persons are not available.

If the theory that high cholesterol levels signify potentially increased hormone production is true, histologic examination should confirm this theory; that it does not is evidenced by the fact that cells which have accumulated excess amounts of lipid do not usually respond to stress with a depletion of lipid as do normal cells. In addition, it is important to note in regard to potentially increased production of hormones that adrenal hypertrophy and high cholesterol values, although frequently found in cases of hypertension, are by no means consistently present.

One of the most pertinent observations concerning the possibility that increased cholesterol values signify repeated stimulation followed by "involution" is that of Ludewig and Chanutin.¹⁶ These authors followed the adrenal cholesterol and ascorbic acid concentrations for seven days in rats subjected to thermal burns and to parenteral injection and

15. Friedman, B.; Somkin, E., and Oppenheimer, E. T.: *Am. J. Physiol.* **128**:481, 1940. Williams, J. R.; Díaz, J. T.; Burch, J. C., and Harrison, T. R.: *Am. J. M. Sc.* **198**:212, 1939. Houssay, B. A., and Dexter, L.: *Ann. Int. Med.* **17**:451, 1942. Goldblatt, H.: *ibid.* **11**:69, 1937.

16. Ludewig, S., and Chanutin, A.: *Endocrinology* **41**:135, 1947.

cutaneous application of nitrogen and sulfur mustards. They noted that after an initial depression the cholesterol regularly rose to supernormal levels, which were still present at the end of seven days. It should also be noted that prolonged administration of an anterior pituitary extract containing the adrenocorticotrophic factor ^{2b} or of crude pituitary extracts ¹⁷ causes an increase of lipid in the adrenal gland, and presumably there was initially depletion of lipid.

Experiments that show marked and rapid fluctuations of adrenal cholesterol, the initial response being depletion, which is later followed by abnormally high levels of cholesterol, form the basis for an explanation of an abnormally high concentration of adrenal cholesterol and the histologic changes that reflect this high concentration.

It seems logical that as the adrenal gland is a dynamic organ its deviations from normal structure and chemical constitution are reflections of periodic activity and "involution." Although "involution" is a term not usually applied to the adrenal cortex, it seems particularly fitting. By analogy one might consider the thyroid gland, in regard to which the term "involution" has had widespread use. For example, when that gland is called on to maintain the homeostatic equilibrium of the body—for instance, in such situations as iodine lack and pregnancy—there follows a series of histologic changes in which hypertrophy, hyperplasia and involution occur. Although during and after this sequence of events there may be no evidence of hyperfunction or hypofunction, the end result is frequently an enlarged gland which is the site of varying histologic patterns and adenomas.

The concept that excess deposits of lipid substances, focal and nodular hyperplasia and small adenomas of the adrenal cortex are the result of cycles of stimulation, depletion and involution may well explain the common occurrence of such lesions in the wide variety and apparently unrelated circumstances in which they are found.¹⁸

Our present interpretation of the abnormally high levels of cholesterol found in some cases of cardiovascular and/or renal disease, particularly those with hypertension, is that the adrenal glands of the patients have been the site of repeated cycles of stimulation and "involution," the end result being morphologic alterations and the storage of excessive amounts of cholesterol. This implies that over a long period of time secretion of adrenal cortical hormones may have been excessive, but it does not imply that adrenal glands containing high levels of cholesterol or adenomas are the site of continual excessive production of cortical hormones such as is believed to occur in Cushing's syndrome.

17. Emery, F. E., and Atwell, W. J.: *Anat. Rec.* 58:17, 1933-34.

18. Moore, R. A.: *A Textbook of Pathology*, Philadelphia, W. B. Saunders Company, 1944, pp. 1068-1069. Goldzieher, M.: *The Adrenal Glands in Health and Disease*, Philadelphia, F. A. Davis Company, 1944, pp. 97-103. Grollman, A.: *The Adrenals*, Baltimore, Williams & Wilkins Company, 1936, p. 337.

SUMMARY AND CONCLUSIONS

The cholesterol contents of 125 pairs of adrenal glands were determined. It was found that when rigid criteria of normality were adopted in selecting material for study, namely, the adrenal glands of persons who suffered sudden death and who at autopsy showed no pathologic changes other than those caused by the fatal agent, the cholesterol values were relatively constant. In 11 normal persons the adrenal cholesterol concentration ranged from 5.35 to 8.20 Gm. per hundred grams of adrenal tissue and the total adrenal cholesterol content from 485 to 720 mg.

Cholesterol values were also determined on adrenal glands from persons who died of cardiovascular and/or renal disease (some having undergone sudden and some prolonged deaths) and from persons who succumbed to infections, cancer and hepatic or endocrine diseases. Included in the endocrine group were 3 patients who had Cushing's syndrome and 1 patient who had the adrenogenital syndrome.

Abnormally high adrenal cholesterol levels were frequently found in persons who died of cardiovascular and/or renal disease. In 4 persons with hyperadrenocorticism the adrenal cholesterol concentration was either low or within normal limits. Persons with infectious diseases and chronic debilitating diseases had low cholesterol values.

In adrenal glands which contained abnormally large amounts of cholesterol three types of cellular change were observed that were the histologic manifestations of increased cholesterol content. It was noted that when an adrenal gland which revealed any one or all of these changes was the site of increased physiologic activity, these abnormal cells did not show depletion of lipid as normal cells do.

An attempt to correlate adrenal cholesterol values with the available data on the excretion of "cortin-like" substances and 17-ketosteroids revealed that when the body has undergone "stress" the excretion of adrenal steroids is high and the adrenal cholesterol content is low. However, low adrenal cholesterol values are not always associated with increased excretion of adrenal steroids, and in hyperadrenocorticism, in which there is sustained excessive production of adrenal hormones, the cholesterol concentration was found to be normal or low. Thus, it would seem that the rate of adrenal hormone production is not necessarily dependent on, or reflected by, the adrenal concentration of cholesterol.

The significance of increases of adrenal cholesterol and the mechanism by which they occur are discussed.

It is suggested that high levels of adrenal cholesterol and their histologic manifestations are the result of repeated cycles of adrenal stimulation and depletion followed by "involution" and storage of excessive amounts of cholesterol.

RETICULUM CELL SARCOMA OF BONE

V. R. KHANOLKAR

Director of Laboratories, Tata Memorial Hospital, and Consulting Pathologist,
King Edward VII Memorial Hospital

BOMBAY, INDIA

THE MAJORITY of primary cancers of bone present an almost hopeless prognosis. Heroic surgical measures, even if started in the early stages, are often unsuccessful in preventing a rapid and fatal termination in osteogenic sarcoma.

Parker and Jackson¹ have, however, isolated a group of primary tumors of bone which present a more hopeful outlook, especially if the conditions are recognized early and treated properly. As a result of a study of 3 tumors of this group of their own and the material from the Registry of Bone Sarcoma of the American College of Surgeons, they were able to report on 17 tumors which formerly "had been variously classified as Ewing's sarcoma, Hodgkin's disease, lymphosarcoma, osteogenic sarcoma, leucosarcoma or as inflammation." They defined the clinical, roentgenologic and histologic characteristics of this group. The Registry of Bone Sarcoma recognized these tumors as a separate entity in 1939.

These neoplasms, in spite of their clearcut characteristics, have not been sufficiently recognized by clinicians, radiologists and pathologists. "The accurate diagnosis of a bone sarcoma remains a rather difficult task. Unless the surgeon and pathologist are familiar with what may happen in the bone, he is hardly able to recognize what has happened."² It is therefore believed that a report of 5 cases observed during the last six years might help to draw greater attention to this interesting group of bone tumors.

REPORT OF CASES

CASE 1.—An emaciated Deccani Hindu, 30 years old, was admitted to Tata Memorial Hospital because of pain in the right thigh. One year before, he had had shooting pains in the hip, which were worse at night. His physician thought that he had tuberculosis of the joint and put the limb in a plaster cast for two months. The treatment did not relieve his pain. His condition became worse. He lost much weight and was unable to walk.

On his entry it was found that the right leg was fixed and incapable of either active or passive movements. Resistance to deep palpation was encountered in the right inguinal region and over the corresponding trochanteric area. A fulness

1. Parker, F., and Jackson, H.: *Surg., Gynec. & Obst.* 68:45, 1939.

2. Ewing, J.: *Surg., Gynec. & Obst.* 68:971, 1939.

was felt in the right lower quadrant of the abdomen as if arising from the pelvic surface of the pubic bone. There was a tenderness on the right side on rectal examination. Roentgen examination (fig. 1) showed a generalized rarefaction of the upper third of the right femur. There was no marginal sclerosis. Similar areas of bone destruction were seen near the pelvic border of the acetabulum, the lower part of the ilium and the ischium on the right side. There was a feeble attempt at a periosteal reaction, as evidenced by the laying down of parallel lamellas of bone in the subtrochanteric portion of the femur. Below the main lesion of the thigh bone the cortex was denser than normal. There was almost complete absence of bone trabeculae in the medullary portion in this region. The dorsal portions of the fourth and fifth ribs on the left side showed similar areas of bone destruction. The blood did not show any significant change from the normal except for mild secondary anemia

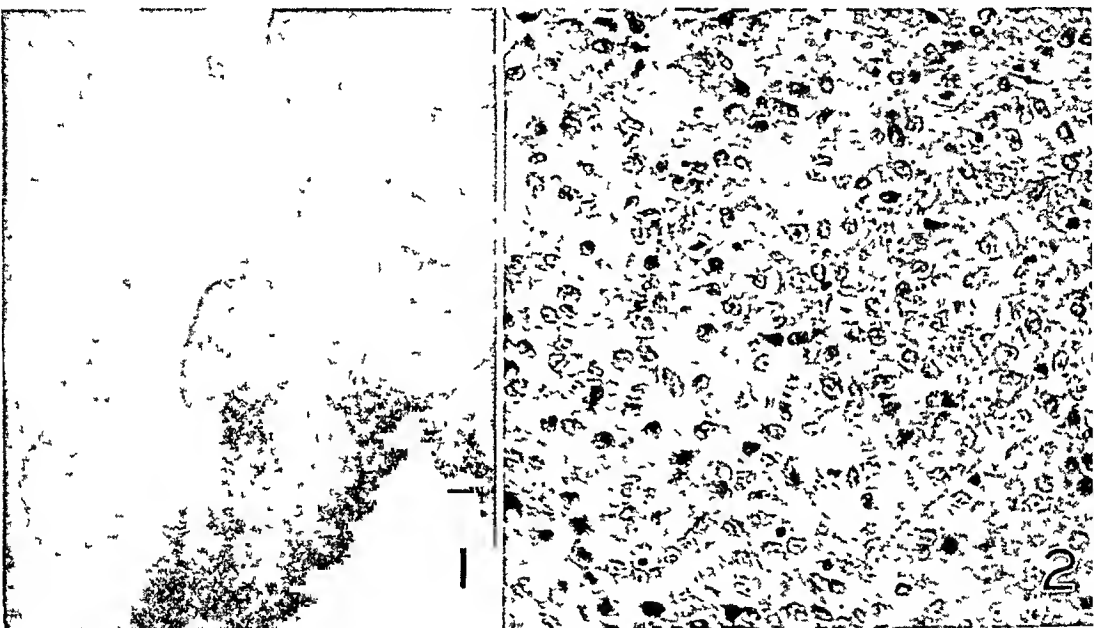


Fig 1 (case 1).—Roentgenogram of the femur and the right side of the pelvis showing changes in the ilium, the ischium and the upper third of the femur.

Fig. 2 (case 1).—Photomicrograph of tissue from the fifth left rib, showing reticulum cell sarcoma. Hematoxylin and eosin stain

There was no Bence Jones protein in the urine

About 4 cm. of the diseased portion of the fifth left rib was resected. The rib was adherent to the pleura posteriorly, and a portion of its wall had been destroyed by disease. The bony tissue was replaced by a grayish soft granular opaque material with irregular rough spicules projecting through it. The histologic examination (fig. 2) revealed the structure of reticulum cell sarcoma (The microscopic characters of the tissue in all the 5 cases were so similar that they have been described later in one place, to avoid repetition)

The patient was treated with roentgen radiation. His general condition remained good, and toward the end of the treatment he was able to move the thigh through 5 degrees. The pain in the thigh and the chest disappeared, and he was able to walk about on crutches

During the first course of radiation a lesion developed in the right scapula, which on roentgen examination showed an elliptic area of rarefaction along the axillary border. After the completion of the course of radiation a piece of the fourth rib was resected from the region previously diseased. On microscopic

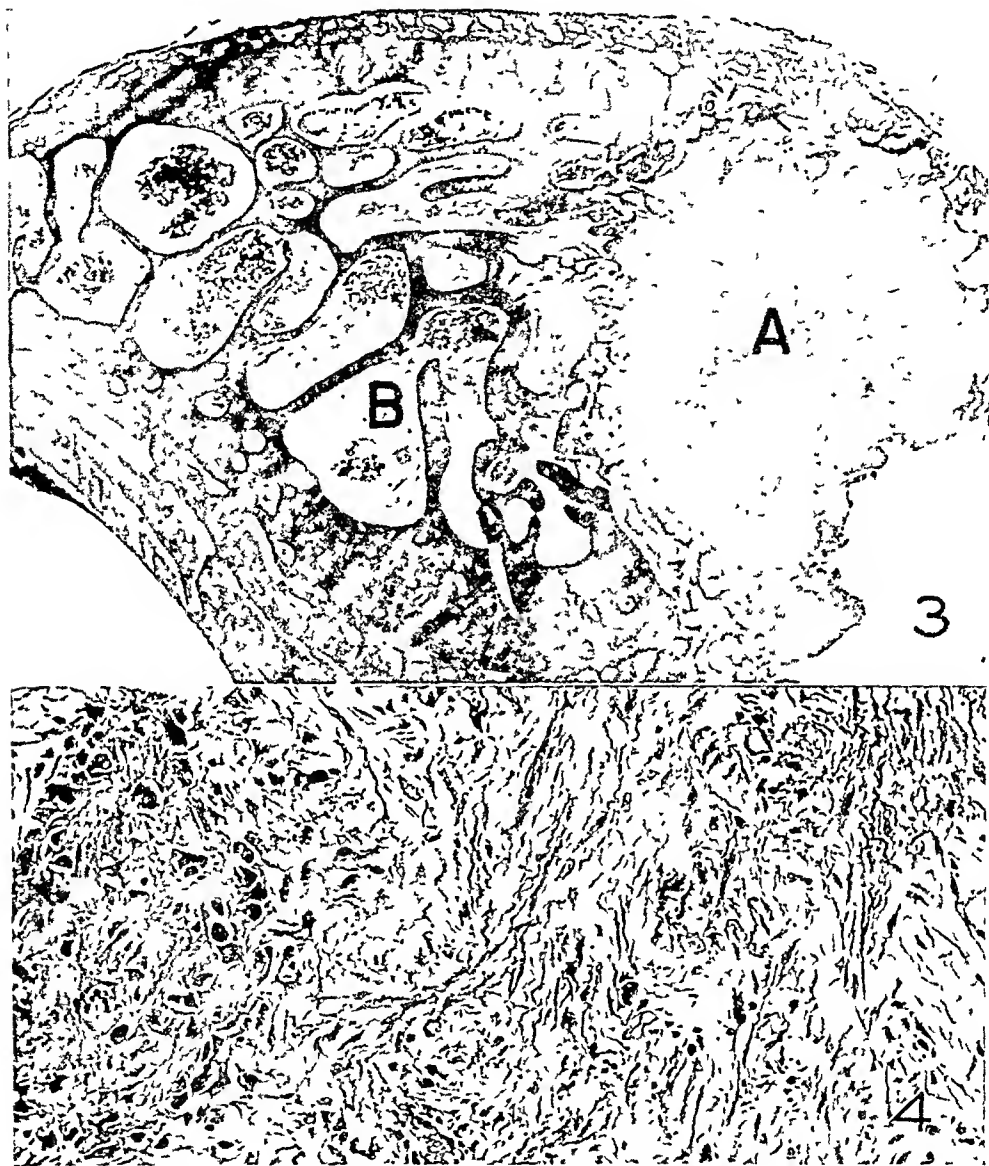


Fig. 3 (case 1).—Transverse section of a rib showing an area in which the bone had been destroyed by sarcoma replaced by fibrovascular connective tissue, marked *A*, and dark staining areas in which fragments of dead bone are seen lying in the marrow, marked *B*. Hematoxylin and eosin stain.

Fig. 4 (case 1).—A higher magnification of the region marked *A* in figure 3 to show the microscopic features of the healed area. Hematoxylin and eosin stain.

examination (fig. 3) it showed not only that the tumor had completely regressed and been replaced by a fibrovascular granulation tissue but also that new trabeculae were being laid down and the bone reproduced. A study of this tissue was

particularly interesting because of the information which it supplied concerning the new bone being formed on the site of a healed tumor.

The patient continued to have some stiffness in the right hip and a little tenderness over the upper part of the right femur for nearly one year. He was gradually able to resume his work and now, three years later, is still alive and well.

CASE 2.—A 20 year old Hindu Deccani farmer was admitted for tumor of the cheeks and inability to swallow solids. He stated that he had noticed painless swelling on the lower jaw some four months previously. It had gradually involved the whole jaw and had spread to both cheeks. Three months later the swelling of the right cheek had burst inside and was discharging foul purulent matter.

There was a large painless swelling occupying almost the whole of the right side of the face. It was firm in front and above, but soft and fluctuating elsewhere. There was a smaller and harder swelling on the opposite side. Inside the mouth, the lower alveolus was thickened by a large elastic nodular growth. The tumor had spread laterally to both cheeks and posteriorly to the anterior pillars of the fauces. It had ulcerated on the right side. The patient was unable to close his mouth. The nodular masses were projecting into the oropharynx and were almost occluding its lumen. There was a hard node in the left submandibular region. The blood showed mild secondary anemia. Blood studies, including determinations of both alkaline and acid phosphatases, gave normal results. A roentgenogram of the mandibles showed multiple rounded areas of rarefaction involving the descending rami on either side of the mandible. A biopsy showed reticulum cell sarcoma.

The patient was started on high voltage roentgen ray therapy and felt so much better after five exposures that he discontinued treatment and went back to his home. He returned after fourteen months. The main bulk of the original tumor had disappeared, but a mass was present over the left maxilla and the parotid gland; there was a swelling also in the region of the tonsils and the soft palate. He was given another course of high voltage roentgen ray therapy. There was rapid regression of the disease. Five months later no disease could be seen on the right side, but there was a brawny swelling in the left preauricular region with softening in the center. This prevented him from opening his mouth or chewing his food. He was further treated with roentgen rays. He was soon able to eat solid food easily, and the pain was relieved. He appeared one month later with a large painful swelling on the left side of his face. The swelling burst through the cheek and discharged large sloughs of necrotic material. Biopsy did not reveal any evidence of neoplastic tissue. The patient's condition rapidly improved, and he left the hospital one week later.

CASE 3.—A 37 year old Rajput cavalryman was referred for a massive swelling of the left shoulder and chest. Six months previously, he had had pain in the left shoulder. Some days later he noticed a swelling in the same region. The swelling gradually increased in size and spread to the armpit and the front of the chest.

He was a well built, athletic man with a large smooth swelling over the upper part of the left side of the chest. There were small firm nodes in the supraclavicular region and a larger mass in the axillary region. The swelling was firm, elastic and slightly painful. There was no fever. A roentgenogram showed that the major portion of the axillary borders of the left scapula was destroyed. There was a huge, soft tissue tumor, which revealed scattered strands of ossification in its substance. The neck of the glenoid process showed condensation of bony trabeculae.

There was slight anemia; otherwise cytologic and chemical analyses showed that the blood was normal. Biopsy specimens taken from the infraclavicular region

showed the characteristics of a reticulum cell sarcoma. The patient was treated with roentgen radiation. The sarcoma began to melt away after three or four treatments, only to appear at some other place on the trunk and the arm. After a couple of months a firm mass developed in the left iliac fossa. The masses regressed with treatment, but new ones cropped up elsewhere. His condition became steadily worse, and because of the low white cell count further irradiation had to be discontinued. The patient died at a military hospital two months after the last radiation treatment. No further information could be obtained.

CASE 4.—A strongly built Moslem, 38 years old, was referred because of a large swelling on the chest. His illness had started with severe asthmatic attacks and precordial pain, eighteen months previously. His condition became steadily worse. There never had been any lymphadenopathy. The white blood cell count was 14,800, with 55 per cent eosinophilic granulocytes. He was therefore believed to have been suffering from Löffler's syndrome, and he was given neoarsphenamine intravenously. His asthmatic symptoms improved dramatically; but a lump developed on the sternum. A couple of months later he began to complain of shooting pains starting in the lower dorsal region, passing down his legs and radiating round his trunk to the epigastrium. The pain appeared only when the patient sat up and bent forward, and was aggravated by coughing. He was treated with high voltage roentgen rays.

About halfway through the treatment a roentgenogram showed marked destruction of the body of the eighth thoracic vertebra. The intervertebral disks were intact. There was a similar but less marked involvement of the first lumbar vertebra.

A swelling 7 cm. in diameter projected about 3 cm. beyond the general outline of the chest over the manubrium. There was a firm node in the right axilla, and a larger softer swelling in the left axilla. There was a small discrete node in the supraclavicular region on the right side, which was excised for examination. The right tonsil was swollen and red but not ulcerated. The patient complained of fleeting pains in the arms and the calves. A roentgenogram of the chest revealed a roughly globular expansion of the manubrium and a well defined small area of rarefaction in the lower segment of the sternum. The excised node showed the characters of a reticulum cell sarcoma.

Owing to the unsatisfactory conditions in the city, the patient decided to go back to his home town and have radiation therapy there.

CASE 5.—A 22 year old Deccani Hindu farmer, well built but extremely emaciated, was admitted to the hospital with retention of urine and inability to move his legs. His complaint had started only six weeks earlier with swelling of the lower jaw and inability to eat. A fortnight later he began to have lightning-like pains in both legs. The pain was much worse at night. This continued for seven days, and on the eighth, as he could not bear the pain any longer, he branded himself with a hot iron. The pain disappeared, but he was unable to move his legs. He became bedridden, and bed sores developed. A few days later he was unable to urinate.

The gums were soft, swollen and spongy. All the teeth were loose. There were marked wasting and loss of movement in both legs. Sensation was absent up to 5 cm. above the symphysis pubis. The knee and ankle jerks were absent. The condition of the patient steadily deteriorated. A fortnight later he died.

Autopsy (summary).—The gums along their whole length in both the maxilla and the mandible were swollen and soft. The color was reddish yellow. Most

of the bony part of the mandible was replaced by a soft growth, which had eroded the bone unevenly. The teeth were loosely embedded in this spongy mass.

The submental, submaxillary and cervical lymph nodes were all moderately enlarged. The spleen was slightly increased in size.

A yellow mass was infiltrating the bodies of the eleventh and twelfth thoracic and the first lumbar vertebrae and the upper part of the psoas muscle in front.

The tumor of the jaws and the spine revealed the characteristics of a reticulum cell sarcoma. The major portion of the tumor tissue was composed of a delicate network of reticulum, the meshes of which were occupied by large cells with pale nuclei. The tumor was traversed by a rich plexus of capillaries and contained areas of hemorrhage and necrosis. There were few remnants of fine spicules of bone in the tumor tissue. The most advanced lesion was seen in the mandible.

GENERAL CONSIDERATIONS

"The existence of a pure reticulum cell lymphosarcoma of bone marrow was recognized many years ago by Kaufmann. He stated that the cells were larger than plasma cells, resembled large or small reticulum cells, with giant cells, and a reticular matrix was present."² However, the recognition of these tumors as a separate clinical entity and a definition of their clinical, roentgenologic and pathologic features is due to Parker and Jackson. The case described by Lef Dahl and Levine³ and case 14 of Craver and Copeland⁴ probably belong to this class. Since Parker and Jackson published their observations, 3 further cases⁵ have been reported in American literature. The reports published in *Acta Radiologica*⁶ were not available. It may now be worth while to review the general features of these tumors on the basis of the reports in the literature and personal observations.

Clinical Aspects.—Primary reticulum cell sarcoma of bone is mainly a disease of younger persons. Parker and Jackson pointed out that whereas in more than three fourths of the cases generalized reticulum cell sarcoma occurs after the age of 40, in about the same proportion of cases reticulum cell sarcoma of bone occurs under that age.

The disease is often ushered in with severe shooting pain referable to the site of the lesion. This was so in 3 of the 5 cases now reported and in 13 of the 17 cases reported by Parker and Jackson. It was also a noticeable feature in the case reported by Edwards and in one of the cases of Szutu and Hsieh.^{5b} The disease progresses slowly in most cases and causes marked destruction of bone. It tends to remain localized for a long time, and a large tumor may be accompanied by

3. Lef Dahl, G., and Levine, V.: Arch. Path. **21**:869, 1936.

4. Craver, L. F., and Copeland, N. M.: Arch. Surg. **28**:809, 1934.

5. (a) Edwards, J. E.: Am. J. Path. **26**:835, 1940. (b) Szutu, C., and Hsieh, C. K.: Ann. Surg. **115**:280, 1942.

6. Eker, R., and Poppe, E.: Acta radiol. **23**:387, 1942. Rosendal, T.: *ibid.* **26**:210, 1945.

few if any metastases. The disease extends into the neighboring muscle and connective tissue and spreads slowly along the lymphatic channels to involve adjacent lymph nodes. There is a predilection for metastases in other bones, sometimes in those of the spine and hardly ever in those of the skull. The most arresting feature is the relative well-being of the patient and his lack of concern about his disease. It was frequently noticed that the attending physician was more alarmed at the extent of the morbid condition than the patient himself. It is necessary to point out that the prognosis of the disease is relatively favorable if it is diagnosed early and prompt measures are adopted toward its cure. The cytologic and biochemical studies in the first 4 cases did not reveal any significant deviations from the normal. In 1 case only the acid phosphatase was higher than normal, but well below the figure in metastatic deposits in cancer of the prostate. The physical signs referable to a disease of bone often raise a clinical suspicion of syphilis, but in the cases of reticulum cell sarcoma the blood is uniformly normal. It is therefore necessary to warn against prolonged anti-syphilitic treatment with a harmful delay of suitable treatment.

Roentgen Diagnosis.—The diagnostic roentgen findings are definite, but not characteristic of the disease. They consist of a mottled or diffuse osteolytic process with little evidence of new bone forming at the periphery of the lesion or under the periosteum. Bone trabeculae appear to melt away as they are involved in the sarcomatous infiltration. The roentgenographic appearances are often confused with those of other tumors or inflammatory lesions of bone. It is likely that with greater appreciation of, and familiarity with, roentgenographic pictures of the disease a correct roentgenologic diagnosis may be expected more frequently.

Structure.—The macroscopic examination of the tumor reveals a grayish soft granular homogeneous tissue dotted with dark reddish areas of hemorrhage and necrosis. There is absence of coarse spicules or trabeculae of bone in most of the tumor mass. The microscopic structure of the tumor is characteristic, as are also the cells which compose it. The tumor tissue is held in a loose fibrovascular framework. The collagen bands are sparse and thin. The intervening space is occupied by a delicate interlacing of argyrophil fibers. These fibers seem to be woven round individual cells or small groups of them. The tumor tissue is richly supplied with blood capillaries. The cells lining the capillaries are often cuboidal, swollen and several layers thick. The tumor cells seem to insinuate themselves between the lining endothelial cells and to push into the capillaries. The vessels present a peculiar petaled appearance in silver preparations. Infarction, degeneration and necrosis occur freely in the tumor. The characteristic cell composing

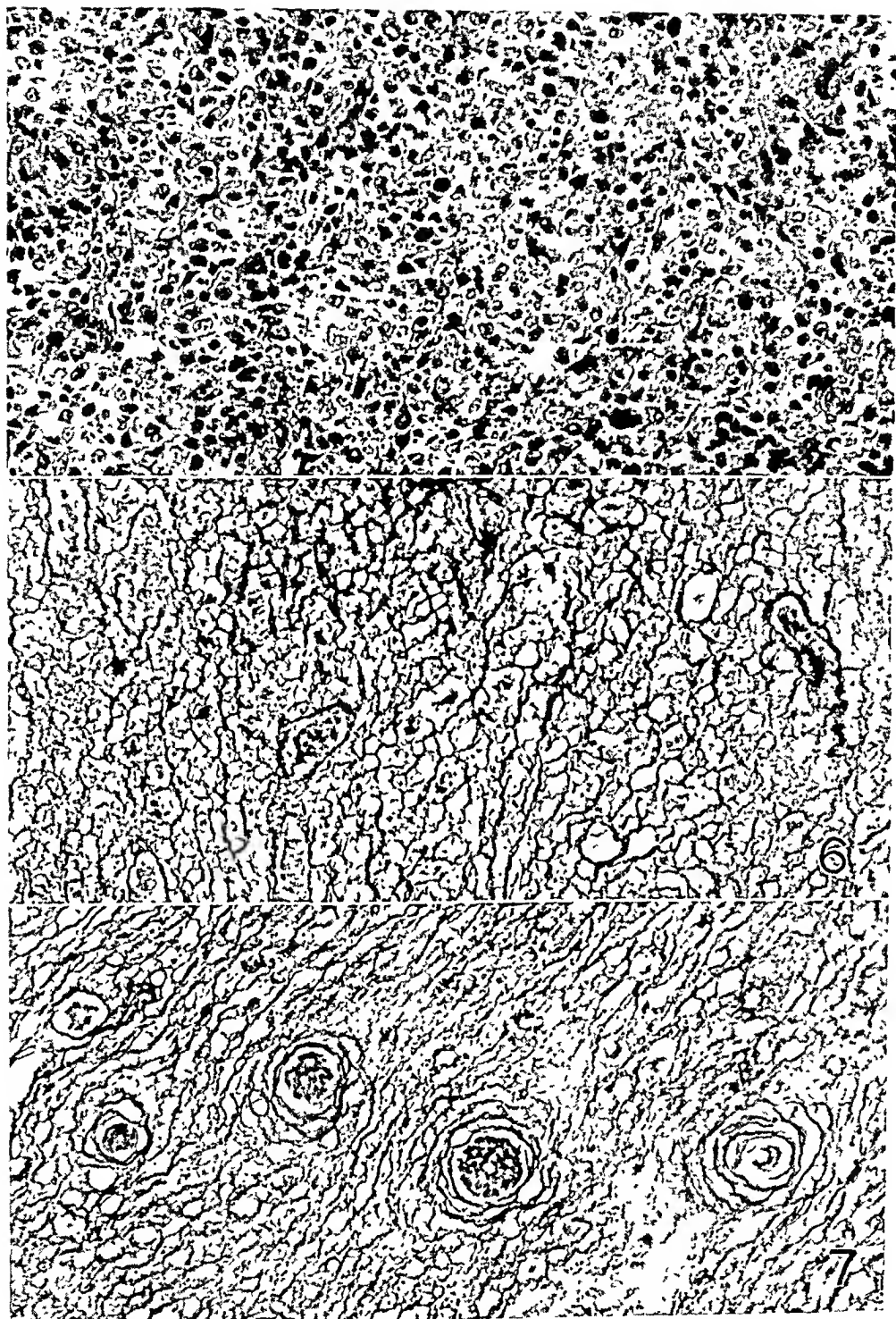


Fig. 5 (case 3).—Photomicrograph of reticulum cell sarcoma. The cells are smaller and more irregular in size and their nuclei are more hyperchromatic than those in case 1. Compare this section with that in figure 2. Hematoxylin and eosin stain.

Fig. 6 (case 1).—Photomicrograph showing the delicate network of reticulin fibers between individual cells or small groups of them. Gömöri reticulum stain.

Fig. 7 (case 1).—High power photomicrograph showing the peculiar petaled appearance of the walls of small blood vessels. Gömöri reticulum stain.

the tumor is a rather large cell, about 20 microns in diameter, with abundant amphophilic or weakly acidophilic homogeneous cytoplasm. The cell borders are vague and irregular. The cells are usually globular, but in some tumors narrow, elongated forms or spheroidal cells with blunt cytoplasmic processes are frequently seen. The nuclei are round, ovoid and occasionally kidney shaped. They are usually large (10 to 12 microns), pale, with distinct nuclear membrane and fine chromatin granules. Some tumors contain cells with a distinct nucleolus. Mitotic figures are infrequent, and atypical mitoses are rare. Large cells or giant cells may occasionally be seen, but they do not resemble Sternberg-Reed cells. In some tumors the cells appear actively phagocytic and contain deeply stained ingested particles. In the degenerating areas few fat droplets are seen in the cell cytoplasm.

Histogenesis.—It is now generally agreed that undifferentiated cells of mesenchymal origin are found in lymph nodes and hemopoietic organs of the adult. These cells possess a rich and varied potentiality to develop into an amazing variety of cells, which may form supporting structures (cartilage, bone and connective tissue), lymphoid, myeloid and myeloblastic tissue, and the reticuloendothelial system of phagocytic cells. In normal health these cells are inconspicuous. They are situated outside and in close apposition to the lining cells of the blood and lymph sinuses. They are pale cells; their outlines are indistinct, and they appear to form a syncytial network. The cells possess faint clear nuclei and are picked out only by their distinct nuclear membrane. They are nonmobile, do not store dyes and are not associated with argyrophilic fibrils. In response to suitable stimulation they become swollen, multiply, move out of their unobtrusive situation and assume a definite cell outline. The nuclei become indented, darker, richer in chromatin and smaller in size. The cytoplasm becomes denser, and fragments of ingested particles can be seen in it. A fine-meshed network of reticulum fibers is laid down in close association with these cells. They bear the usual microscopic characters of cells variously termed as monocytes, clasmatoocytes or reticulum cells. It has been observed that the microscopic appearances, and presumably the physiologic functions, of these cells vary to a certain extent with their location and the environment in which they settle down. The limits of their determination and "the extent of their pluripotentiality" in different environments are still unsettled. It is known, for instance, that cells resembling reticulum cells can give rise to osteoblasts in birds and can revert back to their original morphologic character under certain conditions. It is also known that reticulum cells are abundantly present in normal bone marrow and can be stimulated to increased activity or even neoplastic proliferation under conditions not well understood at

present. It is, however, difficult to understand the reasons for a difference in the activity of their growth in the marrow, as compared to the rapid growth of morphologically indistinguishable cells occurring in lymph nodes and elsewhere.

Therapy.—Reticulum cell sarcoma is strikingly amenable to roentgen radiation. It has even been suggested that this marked sensitivity to radiation may be used as a diagnostic criterion when the tumors are inaccessible to biopsy. The usual dose of high voltage roentgen rays which produces subsidence of a tumor is about 600 r, although some lesions fail to show any evident change until after the dose has reached 800 r. In the primary reticulum cell sarcoma of bone, the improvement following irradiation seems to persist in most cases. Roentgen therapy seems to effect not only a regression of the disease but a condensation of osseous trabeculae and a regeneration of bony tissue in completely destroyed areas. This was observed in cases 1, 2 and 3 of the present report and in the tumor of the scapula described by Szutu and Hsieh. Parker and Jackson observed better results with amputation and irradiation in 8 of the 9 patients so treated. They further found that in 3 cases "in which radiation failed to arrest the disease, subsequent surgical attacks effected an eventual cure."

SUMMARY

Five cases of primary reticulum cell sarcoma of bone observed during the last six years have been described. The necessity for an early recognition of the disease and appropriate treatment of the patients has been emphasized in view of a relatively favorable course of the disease in many cases.

NEOPLASTIC DISEASES OF DOGS

II. Mast Cell Sarcoma, Lymphosarcoma, Histiocytoma

R. M. MULLIGAN, M.D.

DENVER

THIS continuation of the discussion of neoplastic diseases of dogs initiated in a paper on melanoma published elsewhere¹ will include a consideration of the features of mast cell sarcoma, lymphosarcoma and histiocytoma.

REVIEW OF THE LITERATURE

Neoplasms of mast cells, of lymphoblasts and lymphocytes, and of histiocytes will be reviewed so far as they have not been adequately covered in previous papers.²

Mast Cell Sarcoma.—Murray³ was the first to recognize mast cell sarcoma, although earlier workers had listed cases of "round cell sarcoma" without specifying type. He reported 2 cases in which the tumor occurred on a hindleg. Chambers⁴ described mast cell sarcoma in 3 male dogs. The first tumor was on a hindleg of a 9 year old fox terrier, the second in a popliteal space of an 8 year old bull terrier and the third in an axilla of a 10 year old fox terrier. Under the name "mastocytoma," Bloom⁵ recorded 5 instances of mast cell sarcoma. In 4 instances male dogs were involved, the sarcoma affecting the right thigh of a 15 year old Boston terrier, the right superior labial region of a 5 year old English setter, the scrotum, inguinal lymph nodes, spleen, liver, omentum, mesentery and lungs of a 9 year old mongrel spaniel, and the scrotum and inguinal lymph nodes of a 15 year old fox terrier. The female dog was an 8 year old fox terrier with a tumor of the left ear. The finding of heparin in mast cell sarcomas of dogs and the decrease of specific granules accompanying

From the Department of Pathology, University of Colorado School of Medicine.

This investigation was supported by grant C-380R of the National Cancer Institute of the United States Public Health Service.

1. Mulligan, R. M.: Am. J. Path., to be published.

2. Mulligan, R. M.: (a) Arch. Path. **38**:115, 1944; (b) Cancer Research **4**:505, 1944; (c) Arch. Path. **39**:162, 1945; (d) **45**:216, 1948.

3. Murray, J. A.: Third Scientific Report, Imperial Cancer Research Fund, London, 1908, pp. 41-60.

4. Chambers, F.: Vet. Rec. **11**:709, 1931.

5. Bloom, F.: Arch. Path. **33**:661, 1942.

heightened anaplasia of these tumors have been described.⁶ The characteristics of the cells of 2 mast cell sarcomas were investigated by the tissue culture method.⁷

Lymphosarcoma.—Jöhne⁸ found lymphosarcoma of tracheobronchial lymph nodes. Joest observed 4 lymphosarcomas, 1 in mesenteric lymph nodes,^{9a} 2 in the intestines^{9b, c} and 1 generalized.^{9d} Chambers⁴ noted a lymphosarcoma of the pharynx of an 8 year old male golden retriever. Gaiger¹⁰ recorded the case of a golden retriever with a lymphosarcoma involving the intestine, the mesenteric lymph nodes, the liver and one kidney. Jackson¹¹ included 4 cases of lymphosarcoma among 77 instances of tumors in dogs. Bloom and Meyer¹² described 13 cases of lymphosarcoma under the term "malignant lymphoma" and exhaustively reviewed the literature on neoplastic diseases of the lymphoid tissue of dogs.

Histiocytoma.—Although Auler and Wernicke¹³ called this neoplasm "benign round cell sarcoma," Lacroix and Riser¹⁴ "transmissible lymphosarcoma" and I^{2d} "lymphosarcoma," closer study of more cases in this laboratory has led me to designate this tumor as "histiocytoma." Auler and Wernicke¹³ described 60 cases of this type of neoplasm, which was nearly always found in the skin of young dogs, after the age of 6 months, seldom grew larger than 25 mm. in diameter, looked like a sarcoma histologically but did not recur after destruction or extirpation in any case. Of the 52 dogs observed by Lacroix and Riser,¹⁴ 28 were less than 2 years old and 43 were younger than 5 years. The sex incidence was equal. Forty of the dogs were of four breeds: Boston terrier, Scotch terrier, cocker spaniel and fox terrier. Thirty-two tumors involved the skin of the ears, front quarters and face; only 4 tumors were located on the penis or the scrotum, and none affected the vulva or the vagina. The clinical course of the tumors was benign; many of them regressed spontaneously.¹⁵

6. Oliver, J.; Bloom, F., and Mangieri, C.: J. Exper. Med. **86**:107, 1947.

7. Paff, G. H.; Bloom, F., and Reilly, C.: J. Exper. Med. **86**:117, 1947.

8. Cited by Casper, M.: *Ergebn. d. allg. Path. u. path. Anat.* **3** (part 2):754, 1896.

9. Joest, E.: (a) *Ber. u. d. Königl. Tierartzl. Hochschule zu Dresden* **4**:171, 1909; (b) **8**:75, 1913; (c) **14**:58, 1919; (d) **15-16**:42, 1920-1921.

10. Gaiger, S. H.: *Vet. Rec.* **11**:558, 1931.

11. Jackson, C.: *The Onderstepoort Journal* **6**:1, 1936.

12. Bloom, F., and Meyer, L. M.: *Am. J. Path.* **21**:683, 1945.

13. Auler, H., and Wernicke: *Ztschr. f. Krebsforsch.* **35**:1, 1931.

14. Lacroix, J. V., and Riser, W. H.: *North American Vet.* **28**:451, 1947.

15. Material from cases of mast cell sarcoma, lymphosarcoma and histiocytoma was contributed by veterinarians listed previously¹ and also by the following veterinarians: G. S. Bolton, W. L. Brayley, A. N. Carroll, H. C. Evers and W. B. Teller, W. K. Fauks, E. C. Jones, E. Laitinen, and G. G. Miller, Jr. Technical assistance was as indicated in the first of these publications.¹

MAST CELL SARCOMA

Analysis of Cases.—The 47 cases of mast cell sarcoma studied have been summarized in table 1. Of the 45 dogs with mast cell sarcoma whose age was known, 5 were 2 to 5 years old, and the remaining 40 were 6 to 14 years old. The sex was known in 46 dogs; 29 were female and 17 were male. Among the 47 cases, the breeds represented included the following: terrier, 29 (Boston, 19; fox, 6; mixed, 3; Manchester, 1); mongrel, 3; shepherd, 3; setter, pointer, dachshund, cocker spaniel, 2 each, and boxer, great Dane, Chesapeake Bay retriever and poodle, 1 each. Noteworthy was the high incidence of mast cell sarcoma affecting Boston terriers; of the 19 studied, the sex of 14 was female and that of 5 male. Information concerning the location of the primary neoplasm was available in 47 cases: A posterior extremity was involved in 12; the scrotum, in 9; a flank in 4; the vulva, an axilla, an eyelid and an anterior extremity, in 3 each; a mammary gland and the neck in 2 each; an ear, the penile sheath, a lip, the perineum, the abdomen and the sternal region, in 1 each.

Gross Findings.—Of the 47 mast cell sarcomas, 43 were solitary and 4 were multiple. This group constituted over 8 per cent of the neoplasms studied in this laboratory. Ten were observed at autopsy. 36 were excised surgically, and 1 was studied at both operation and necropsy. Among the 10 cases in which autopsies were made, regional lymph nodes were involved in 7 and the spleen in 2. In 1 surgical case regional lymph nodes were affected.

The size of the primary neoplasm was known in 38 cases; it varied from 216 cu. mm. (6 by 6 by 6 mm.) to 576,000 cu. mm. (90 by 80 by 80 mm.). In 12 cases the volume was 9 cc. or less; in 20, 10 to 200 cc., and in 6, 200 to 600 cc. The primary neoplasm and overlying skin were often ulcerated. The cut section was usually firm and lobulated or whorled. The consistency was also described as fairly firm, elastic, friable, cystic or porous or spongy, focally softened or focally gelatinous. The color was variously noted as gray, pink-gray, white, gray-yellow or light tan and, in some cases, yellow streaked, red stippled or dark red mottled (fig. 1).

Microscopic Structure (figs. 2 to 6).—With the hematoxylin and eosin stain, the neoplastic mast cells of the more mature tumors, which tended to be relatively small grossly, were polyhedral and had discrete or sharply demarcated borders, although a stubby spindle shape resulted when they were caught among bundles of collagen fibrils. The cytoplasm was pale, acidophilic and abundant when compared with the size of the nucleus. Some cells showed a suggestively vacuolated cytoplasm. Most of the cells contained numerous coarse, dark blue granules, in many to the point of obscuring the nuclei. The nuclei

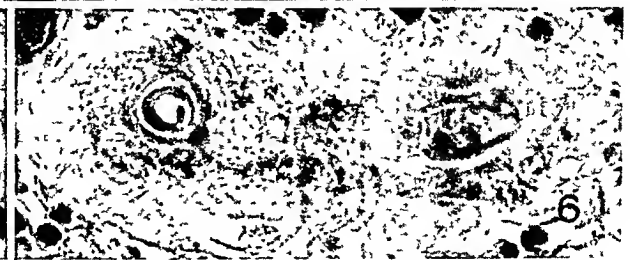
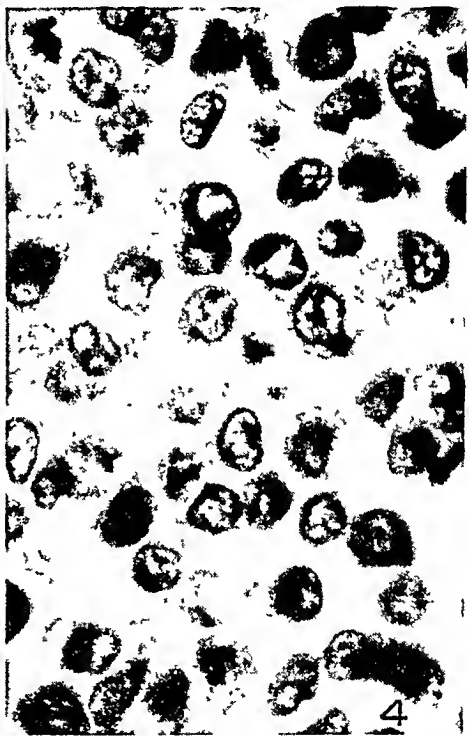
TABLE 1.—*Summary of 47 Cases of Mast Cell Sarcoma*

Case	Age of Dog, Yr.	Sex	Breed	Location
1	13	F	Fox terrier	Left flank, inguinal lymph nodes
2	9	F	Boston terrier	Left tibial region, thigh, flank
3	3	F	Boxer	Nipple, left fourth mammary gland
4	4½	F	English setter	Right tarsal region
5	12	M	Fox terrier	Left gluteal region
6	9	F	Boston terrier	Vulva
7	8½	F	Boston terrier	Right axillary region
8	10	M	Boston terrier	Scrotum
9	10	F	Boston terrier	Left gluteal region
10	12	M	Boston terrier	Scrotum, inguinal lymph nodes, spleen
11	5	F	Great Dane	Right stifle
12	8	M	Fox terrier	Left flank
13	7	M	Chesapeake Bay retriever	Scrotum
14	14	F	Mongrel terrier	Right dorsal cervical region
15	9	M	Boston terrier	Hindleg
16	14	M	Mongrel terrier	Scrotum
17	12	M	Wire-haired fox terrier	Ear
18	9	F	Pointer	Vulva
19	4	F	Poodle	Left hindleg
20	7	F	Boston terrier	Left flank
21	7	F	Boston terrier	Left flank
22	12	..	Shepherd	Axilla
23	10	M	Mongrel shepherd	Prepuce, penile sheath, inguinal lymph nodes
24	7	F	Boston terrier	Prescapular region
25	8	M	Irish setter	Hindleg, lymph nodes, spleen
26	14	M	Mongrel	Eyelid
27	Old	M	Mongrel	Scrotum
28	11	M	Boston terrier	Scrotum, penile sheath
29	10	F	Dachshund	Upper lip, regional lymph nodes
30	8	F	Boston terrier	Right eyelid
31	14	M	Boston terrier	Scrotum, inguinal lymph nodes
32	Old	F	Boston terrier	Perineum
33	10	F	Boston terrier	Left carpal region, axillary lymph nodes
34	11	F	Mongrel fox terrier	Right thigh
35	12	M	Fox terrier	Scrotum, inguinal lymph nodes
36	11	F	Dachshund	Vulva
37	7	F	Cocker spaniel	Sternal region
38	7½	F	Boston terrier	Right upper eyelid
39	14	M	German shepherd	Right thigh
40	8	F	Mongrel	Foreleg
41	11	F	Boston terrier	Mammary gland
42	13	M	Mongrel terrier	Scrotum
43	12	F	Cocker spaniel	Left forearm, axillary lymph nodes
44	10	F	Manchester terrier	Thigh
45	7	F	Pointer	Left axilla
46	2	F	Boston terrier	Right gluteal region
47	13	F	Boston terrier	Abdomen

were round or slightly oval and most often centrally located, but occasionally were eccentric. Sometimes they were dark and condensed. The perinuclear membrane was fairly heavy but discrete and uniform. The chromatin was fine, homogeneous and evenly distributed. Rare binucleated cells were observed, and mitotic figures were unusual or absent. The nuclei were fairly uniform in size and were occasionally reniform. Nucleoli were small, discrete and usually single, but rarely were double.

In the less differentiated neoplasms, studied with the hematoxylin and eosin stain, the borders of the neoplastic cells tended to be fuzzy; the cytoplasm was pale to moderately basophilic and was relatively small in volume in relation to the size of the nucleus. Sometimes the volume of the nucleus was so great as to leave but a narrow rim of cytoplasm, with the result that the cell resembled a lymphoblast. Eccentricity and multiplicity of nuclei in some cells reminded of plasmablasts. The cytoplasmic granules were pale purple, few and fine, or occasionally coarse, dark blue and fairly abundant, or, most commonly, could not be demonstrated except by Giemsa or Nissl stains. Even these two stains showed but few specific granules in the cells of two neoplasms studied. Because of the relatively few granules, the nuclei were conspicuous, rounded and enlarged to varying degrees. Some nuclei were quite irregular and warped into bizarre shapes. Giant nuclei were occasionally seen, especially in multinucleated tumor cells. Some cells contained two or more, occasionally as many as seven, nuclei. Mitotic figures were irregular and varied from a few to many. The chromatin was irregular, coarse and more abundant at the periphery of the nucleus. Especially in multinucleated tumor cells the chromatin was excessively heavy. Nucleoli were enlarged and prominent and sometimes numbered two or three to a nucleus. The features of anaplasia were definitely more pronounced in recurrent tumors.

The neoplastic mast cells were arranged in groups or were scattered singly and often were separated by irregular bundles of increased collagenous connective tissue. The proportions of neoplastic mast cells and connective tissue varied. Broad bands of collagenous connective tissue were intermingled with scattered tumor cells, or large groups of tumor cells were mixed with isolated bundles of collagen fibrils, or solid masses of tumor cells showed no related connective tissue. Locally the neoplastic cells were present within lymphatic channels and veins and invaded skeletal muscle. In lymph nodes the neoplastic cells infiltrated sinusoids, trabeculae and lymphoid cords. In the spleen they were enclosed within sinusoids, usually in those adjacent to the lymphoid nodules.



(See legend on opposite page)

The stroma of increased collagenous connective tissue contained scattered or many segmented neutrophils, sometimes to the point of formation of miliary abscesses. The connective tissue stroma was infiltrated less abundantly in some cases by lymphocytes and also by scattered eosinophils and plasma cells. Mature blood cells, especially neutrophils, were also mingled with tumor cells. Areas of myxomatous degeneration of the connective tissue were fairly frequent. Abundant granulation tissue mingled and merged with the connective tissue stroma near the surface of ulcerated neoplasms. Areas of edema and necrosis affected masses of neoplastic cells and stroma both in the local neoplasm and in affected regional nodes in several tumors. Heavy deposits of hyalin encompassed blood vessels in an occasional tumor.

When intact, the epidermis of the overlying skin was diffusely thinned or was thick and acanthotic in small areas. Most often the epithelium was destroyed and the ulcerated surface was covered with fibrinopurulent exudate, which blended with underlying abundant granulation tissue. In ulcerated tumors the epidermis next to the ulcers often showed increased thickness, lengthened and broadened rete pegs, acanthosis, and mitotic figures in the deepest layers. Cystic and atrophic sebaceous and sweat glands were numerous and intimately associated with the tumor and its supporting stroma in several neoplasms, thus explaining the porous, spongy or cystic appearance noted grossly.

LYMPHOSARCOMA

Seven illustrative cases are reported:

CASE 1.—A spayed female Boston terrier, 9 years old, had a lymphoblastic lymphosarcoma of the cervical, tracheal, mediastinal, mesenteric and inguinal lymph nodes, the spleen, the liver, the bone marrow and the lungs. The lymph nodes ranged between 17 and 22 mm. in greatest diameter. A pink-gray nodule, fairly soft, measuring 2 cm., involved the upper pole of the spleen. The blood contained: hemoglobin, 14.2 Gm. per hundred cubic centimeters; erythrocytes,

Fig. 1 (case 9, table 1).—Mast cell sarcoma. A cut section is shown at the top; the line of excision from surrounding tissues, at the bottom. Skin partly covers both portions. $\times 4/5$.

Fig. 2 (case 11, table 1).—Mast cell sarcoma. An ulcerated area is seen in the left upper corner. Note the hyperplastic epidermis at the surface. The tumor pattern suggests stippling, each black dot representing a neoplastic mast cell. $\times 50$.

Fig. 3 (case 11, table 1).—Neoplastic mast cells with many specific granules. $\times 1,000$.

Fig. 4 (case 12, table 1).—Neoplastic mast cells with few or no specific granules. $\times 1,000$.

Fig. 5 (case 8, table 1).—Neoplastic mast cells with giant bizarre nuclei. $\times 1,000$.

Fig. 6 (case 22, table 1).—Mast cell sarcoma showing deposit of hyalin around a blood vessel. $\times 450$.

(All these photomicrographs were taken from tissue sections stained with hematoxylin and eosin.)

6,250,000 per cubic millimeter; leukocytes, 14,300 per cubic millimeter, with stab neutrophils 7, segmented neutrophils 77, lymphocytes 14 and normoblasts 2 per cent.

CASE 2.—A male Scotch terrier, 9 years old, had a lymphoblastic lymphosarcoma in the cervical, mediastinal and mesenteric lymph nodes (11 to 21 mm. long), the spleen, the liver and the lungs. The bone marrow showed diffuse hyperplasia. The blood contained: hemoglobin, 11.3 Gm. per hundred cubic centimeters; erythrocytes, 5,330,000 per cubic millimeter; leukocytes, 12,050 per cubic millimeter, with stab neutrophils 17, segmented neutrophils 63, lymphocytes 18, monocytes 1 and normoblasts 1 per cent.

CASE 3.—A spayed female Scotch terrier, 9 years old, suffered from generalized lymphadenopathy, dyspnea and anorexia for seven months. Autopsy disclosed a lymphocytic lymphosarcoma in the cervical, tracheobronchial, mediastinal, mesenteric and inguinal lymph nodes, the spleen, the liver, the bone marrow, the gallbladder, the stomach, the kidneys, the lungs and the thyroid and parathyroid glands. Two agonal ulcers marked the antral mucosa of the stomach. The thyroid gland showed diffuse hyperplasia.

CASE 5.—A female collie, 3 years old, showed anorexia, loss of weight and diarrhea for three months before death. Autopsy disclosed in the first 8 cm. of the colon a diffuse pink-gray soft infiltration of the wall, so that the outside diameter of the colon was 6 cm. and the lumen was narrowed to an irregular slit. In the mesentery of the intestines masses of pink-gray soft lymph nodes measured 16 by 12 by 10 cm., and in the anterior mediastinum similar masses measured 10 by 8 by 7 cm., with single nodes 2 to 5 cm. in greatest diameter. The right lobes of the liver contained two umbilicated round pink-gray nodules, 4 and 5 cm. in diameter. The microscopic diagnosis was lymphoblastic lymphosarcoma.

CASE 6.—A male cocker spaniel, 9 years old, had anorexia, emaciation, generalized lymphadenopathy and dyspnea for two months before death. At autopsy, lymphoblastic lymphosarcoma involved the submaxillary, cervical, tracheobronchial, mediastinal, mesenteric, retroperitoneal, iliac and inguinal lymph nodes, the spleen and the liver. The cervical lymph nodes ranged to 9 cm. in length, the submaxillary to 4 cm., and the remaining nodes ranged between 2 and 7 cm. The bone marrow showed myeloid hyperplasia and was not infiltrated by specific cells.

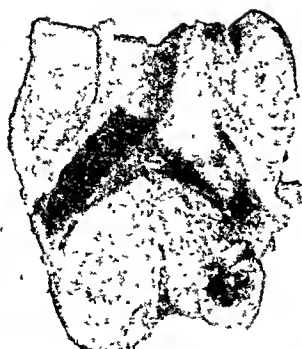
CASE 7.—A spayed female Scotch terrier, 10 years old, showed generalized lymphadenopathy, anorexia, loss of weight and respiratory distress for three months before an enlarged right prescapular node was subjected to biopsy, which established the diagnosis of lymphocytic lymphosarcoma. The blood smear showed: stab neutrophils, 5 per cent; segmented neutrophils, 40.5 per cent; lymphocytes, 45.0 per cent; eosinophils, 8.5 per cent; monocytes, 0.5 per cent, and normoblasts, 0.5 per cent. One gram of urethane U. S. P. was given intramuscularly daily for thirty-five days, but the superficial lymph nodes continued to enlarge and the symptoms progressed. Autopsy disclosed unaltered lymphocytic lymphosarcoma in the 2 to 5.5 cm. submaxillary, cervical, tracheobronchial, mediastinal, mesenteric, prescapular and popliteal lymph nodes and in the liver, the bone marrow, the kidneys, the stomach and the parathyroid glands. The Scotch terrier described in case 3 was a litter mate. A male of the same litter had similar clinical findings before death, at the age of 7 years, but no autopsy was made.

Analysis of Cases.—The 16 cases of lymphosarcoma accounted for about 2.5 per cent of the neoplasms studied in this laboratory. In 15 the age was available; 3 dogs were 3 to 5½ years and 12 were 6 to 12 years old. The sex of the 16 dogs was male in 5 and female in 11 (6 intact, 5 spayed). The following breeds were represented: terrier, 11 (Scotch, 5; Boston, 2; fox, 2; English bull, 1, unspecified, 1); spaniel, 4 (cocker, 3; English springer, 1), and collie, 1. The tissues studied were surgically excised in 4 cases, obtained at autopsy in 11, and were studied at both operation and necropsy in 1 case. Lymph nodes were involved in all 16 cases. In 12 cases all lymph nodes (submaxillary, cervical, tracheal, bronchial, mediastinal, mesenteric, retroperitoneal, iliac, axillary, prescapular, inguinal) were affected. In 4, local lymph nodes were involved, submaxillary in 2 and cervical in 2. Among the 12 cases in which autopsies were made, the spleen was involved in 9 of 10 in which it was examined, the liver in 9 of 10, the kidneys in 4 of 8, the colon in 2 of 7, the lungs in 3 of 6, the stomach in 2 of 6, the bone marrow in 3 of 5, the parathyroid glands in 2 of 5, the thyroid gland in 1 of 5 and the gallbladder in 1 of 5.

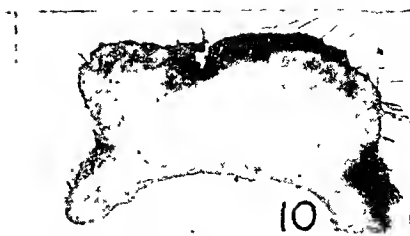
Gross Findings.—The enlarged lymph nodes ranged from 1 to 9 cm. in greatest diameter, were soft or elastic or semiliquid, and pink-gray or tan (fig. 7). The spleen was enlarged, soft, bulging on section and marked by many rounded gray areas 0.5 to 2 mm. in diameter. One spleen contained a 2 cm. pink-gray soft nodule. The liver was of normal size or moderately enlarged. The periportal areas were outlined by gray dots and irregular gray streaks. One liver showed two pink-gray soft nodules, 4 cm. and 5 cm. in diameter, in the right lobes. The stomach, the colon and the gallbladder showed diffuse or localized gray thickening of the mucosa or the wall or of both. The kidneys, the lungs, the thyroid gland and the parathyroid glands were not remarkable grossly.

Microscopic Examination (figs. 7 to 9).—Of the 16 cases studied, 14 were of the lymphoblastic type and 2 of the lymphocytic type.

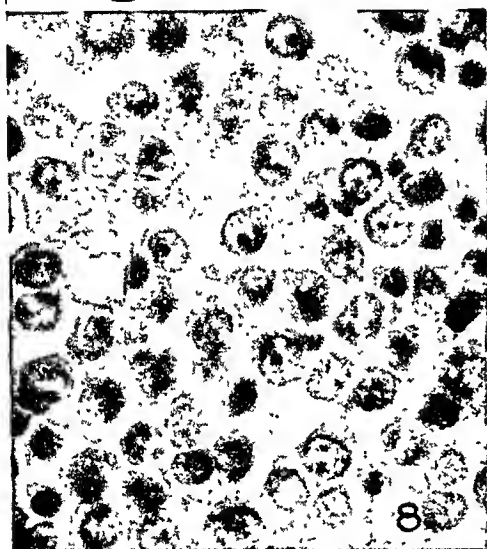
With the hematoxylin and eosin stain, the lymphoblast had a polyhedral or an undulated or scalloped border and fairly plentiful polychromatophilic or pale acidophilic cytoplasm. The nuclei were usually round or oval, sometimes reniform, and often irregular. The chromatin was coarse, unevenly distributed, and clumped together most abundantly at the edge of the nucleus. The heavy nuclear membrane was sharply defined. Nucleoli were large, prominent, acidophilic, and numbered one or two, sometimes three, in a nucleus. Mitotic figures were fairly numerous. Cells intermediate in development between lymphoblasts and lymphocytes were intermingled. Mature lymphocytes were also scattered among masses of lymphoblasts.



7



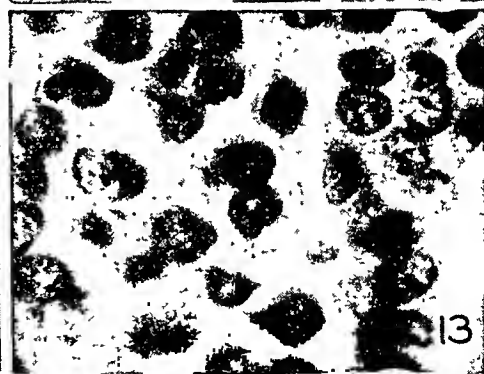
10



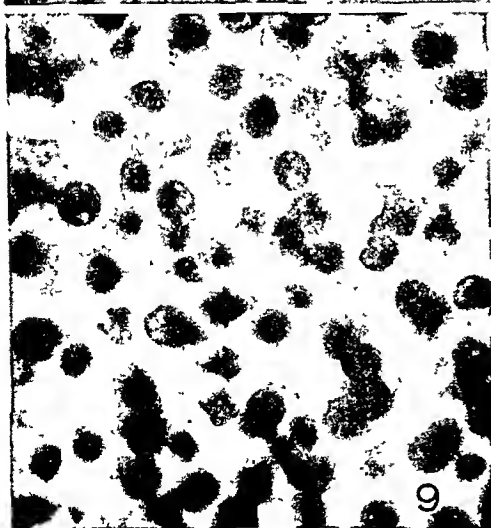
8



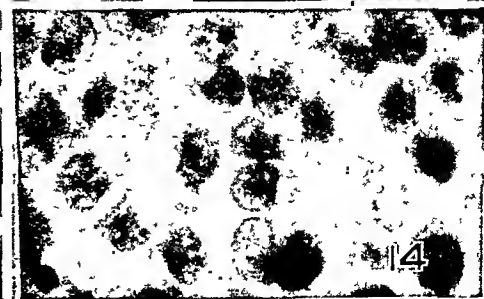
12



13



9



14

(See legend on opposite page)

The lymphocyte was round and discrete and had a pale acidophilic cytoplasm, scanty to fairly abundant. The nuclei were round or slightly oval and sharply demarcated from the cytoplasm. The chromatin was heavy, condensed and frequently was gathered in a solid mass throughout the nucleus. In some nuclei the edges of nucleoli, largely obscured by condensed chromatin, were suggestively visible. Mitotic figures were absent. Scattered lymphoblasts were mingled with masses of lymphocytes.

With Giemsa stain, the cytoplasm of the lymphoblast was moderately to slightly basophilic, and that of the lymphocyte, pale basophilic. The nucleoli of the lymphoblast were deep basophilic.

Regardless of the type of cell proliferated, the lymph node involved showed its components rather monotonously inundated by lymphoblasts or by lymphocytes. The lymphoid nodules were absent as such, and at their former sites cells were blended imperceptibly with the lymphoid cords. The sinusoids were filled with specific cells, so that the borders between cords and sinusoids were obliterated or obscured and the node became one mass of proliferated cells. In the background were scattered lymphocytes with cytoplasmic bodies intact but with nuclei broken into deeply basophilic round fragments. In the background was noted also a fine sprinkling of reticuloendothelial cells, foamy macrophages, plasma cells, and phagocytes enclosing nuclear fragments. The capsule of the lymph node, the surrounding adipose and connective tissues, and occasionally skeletal muscle were infiltrated by specific cells.

In the spleen the lymphoid nodules were composed of specific cells, were greatly enlarged and were fused together. The red pulp was more or less infiltrated and obliterated. In the liver the periportal areas were infiltrated by specific cells and were widened to a varying degree. In the lungs the lymphoid nodules were composed of specific cells and were increased in both size and number. In the kidneys infiltrations were noted in the stroma of the cortex and in the peripelvic adipose tissue. All coats of the colon were diffusely involved. The mucosa of the stomach was affected. The specific cells widely flooded the bone

Fig. 7 (case 6).—Lymphosarcoma. A cut section of an enlarged cervical lymph node is shown at the left, and one of hypertrophied tracheobronchial lymph nodes, at the right. $\times 7/10$.

Fig. 8 (case 13).—Neoplastic lymphoblasts from a lymph node. $\times 1,000$.

Fig. 9 (case 3).—Neoplastic lymphocytes from a lymph node. $\times 1,000$.

Fig. 10 (case 21, table 2).—Histiocytoma, cut section. Note the ulcerated skin surface at the top. $\times 1\frac{1}{3}$.

Fig. 11 (case 21, table 2).—Histiocytoma. An ulcerated surface is shown at the top, with hyperplastic epidermis and hair follicle beneath and to right, surrounded by closely packed neoplastic histiocytes. $\times 35$.

Fig. 12 (case 7, table 2).—Neoplastic histiocytes in skin. $\times 1,000$.

Fig. 13 (case 19, table 2).—Neoplastic histiocytes in skin. $\times 1,000$.

Fig. 14 (case 8, table 2).—Neoplastic histiocytes in skin. $\times 1,000$.

(All these photomicrographs were taken from tissue sections stained with hematoxylin and eosin.)

marrow by displacing the usual granulocytic, erythrocytic and megakaryocytic elements. All coats of the gallbladder were infiltrated. In the parathyroid glands the specific cells crowded between cords of parenchymal cells. Small foci of specific cells marked the stroma between follicles of the thyroid gland. No leukocytes diagnostic of leukemia were found in the peripheral blood in the 4 cases in which it was examined.

HISTIOCYTOMA

Analysis of Cases.—Among 21 cases studied, and summarized in table 2, 7 involved dogs less than 1 year old, 7 dogs 1 to 2 years old, 5 dogs 2 to 5 years old, and 2 dogs over 6 years old. The sex was male in 15 and female in 6. The following breeds were represented: terrier, 9 (Boston, 4; fox, Scotch, American bull, Kerry blue, and unspecified, 1 each); spaniel, 8 (cocker, 7; English springer, 1), and poodle, shepherd, English setter and boxer, 1 each.

The location of 20 neoplasms was as follows: anterior extremity, 6; posterior extremity, 4; ear, face, axilla and thorax, 2 each; penile sheath and vagina, 1 each. On the extremities, the carpal and metacarpal regions, the elbow, the hock and the stifle were affected.

Gross Findings.—Without exception, all 21 tumors studied were excised surgically. They constituted about 3.5 per cent of the neoplasms examined in this laboratory. They ranged in size from 288 cu. mm. (8 by 6 by 6 mm.) to 15,246 cu. mm. (42 by 33 by 11 mm.). Three were less than 900 cu. mm. in volume, 8 were 1,000 to 1,800 cu. mm., 6 were 2,000 to 5,000 cu. mm., 3 were 8,000 to 12,000 cu. mm. and 1 was 15,000 to 16,000 cu. mm.

Grossly, the tumors were often ulcerated, firm, white or light gray, usually homogeneous, occasionally finely nodular and sometimes red stippled or dark red mottled (fig. 10).

Microscopic Examination (figs. 11 to 14).—With the hematoxylin and eosin stain, the neoplastic histiocytes had abundant, finely granular, acidophilic or polychromatophilic cytoplasm with an indefinite, undulated, wavy border and with frequent overlapping of adjacent cells. The nuclei were usually round or oval but were also oblong, reniform, C shaped, wrinkled or even shapeless. The nuclei varied considerably in size. Occasional binucleated forms were observed. The nuclear membrane was thin and distinct. The evenly distributed chromatin was threadlike or filamentous or finely beaded. The nucleoli were small, acidophilic, usually single and occasionally double. Many regular mitotic figures were seen. In some nuclei the chromatin was partly condensed and shifted toward the nuclear periphery; nucleoli were enlarged and prominent, numbering two or three in some nuclei. Such nuclei were quite plentiful in one tumor which contained many small

areas of coagulation necrosis. The neoplastic cells infiltrated widely throughout the dermis and hypodermis and frequently crowded around hair follicles, sebaceous glands and sweat glands. Bundles of collagen were intermingled with masses of tumor cells. Scattered lymphocytes and occasional mast cells, plasma cells and segmented neutrophils were also interspersed among the tumor cells. In some tumors, lymphocytes were present in solid aggregates or in palisaded rows. The borders of the tumors merged indefinitely with surrounding, unaffected connective tissue stroma. A few small areas of coagulation necrosis marked some tumors.

TABLE 2.—*Summary of 21 Cases of Histiocytoma*

Case	Age of Dog, Yr.	Sex	Breed	Location
1	12	M	Boston terrier	Right thoracic region
2	3½	F	Cocker spaniel	Left metacarpal region
3	12	M	Cocker spaniel	Left carpal region
4	15	F	Cocker spaniel	Right axillary region
5	24	M	Scotch terrier	Right metacarpal region
6	60	F	Cocker spaniel	Left ear
7	7	M	American bull terrier	Left stifle
8	45	M	Kerry blue terrier	Left foreleg
9	4	F	Cocker spaniel	Right axilla
10	45	M	Poodle	Prepuce
11	3	M	Mongrel shepherd	Left ear
12	4	M	Cocker spaniel	Skin
13	24	M	Boston terrier	Right hock
14	7	M	Cocker spaniel	Left pectoral region
15	105	F	Boston terrier	Left elbow
16	12	M	Boxer	Supraorbital region
17	18	M	Boston terrier	Right carpal region
18	5½	M	English setter	Left hock
19	96	M	Fox terrier	Left buccal region
20	18	F	English springer spaniel	Vagina
21	18	M	Terrier	Left stifle

With Giemsa stain, the cytoplasm of the neoplastic histiocytes was pale to moderately basophilic but contained no granules. The nucleoli were basophilic. With Foot's modification of Bielschowsky's stain, reticulum fibrils surrounded groups of tumor cells but were not intimately associated with single cells, an observation indicating that these fibrils were present only as part of the supporting stroma and were not formed by the cells themselves. With sudan IV stain, the tumor cells did not contain tingible lipid. This tended to show that the neoplastic cells may have been stimulated to proliferation by antigens rather than by fatty substances infiltrating their cytoplasm. The cells of the 19 extra-genital neoplasms were identical with those of the 2 observed in the penile sheath and the vagina.

In addition to reticulum, the stroma consisted of varying amounts of collagenous connective tissue. Areas of edema were noted in some tumors.

The epidermis was often ulcerated and replaced by a crust of fibrinopurulent exudate, which was focally necrotic and marked by masses of bacteria, seen as cocci with Giemsa stain. Granulation tissue was minimal or absent at the ulcerated surface. When intact, the epidermis sometimes was thinned, and the rete pegs were flattened; at other times the intact epidermis, thrown into broad folds, had a thick corneal layer and showed acanthosis, elongated and broadened rete pegs and scattered mitotic figures. Sometimes vesicles and pustules marked the epidermis.

DEFINITION OF TERMS

The term "sarcoma," deeply ingrained in the literature is suitable for labeling any cancer of mesenchymal origin, even though it means simply "fleshy tumor." Since mesenchyme is generally accepted as the parent tissue of mast cells, lymphoblasts and lymphocytes, the term "mast cell sarcoma" has been used to designate neoplasms of mast cells, and the term "lymphosarcoma," neoplasms of the cells of the lymphoid series.

"Mast cell sarcoma" is an appropriate name for the neoplastic growths of mast cells by priority,³ if for no other reason. Murray³ found that some mast cell sarcomas were "very malignant" but that others were "practically benign." Bloom⁵ also observed variation in the degree of "malignancy" of neoplasms of mast cells. Microscopic gradations of anaplasia have been observed in the neoplasms of mast cells in the 47 cases described. The rapid growth of these tumors, which usually attain large dimensions in a few months, although they may have grown relatively slowly for as long as a year previously; the difficulty of defining the limits of a given tumor at operation so that enough tissue is resected to ablate it; the strong tendency to recur (one to three times); the increasing size and wider spread of the tumor with recurrence; the heightened signs of anaplasia of the neoplastic mast cells in the recurrent lesions; the presence of multiple tumors in about 8 per cent of cases, and the proved involvement of regional lymph nodes in over 15 per cent of cases—all these features argue in favor of "mast cell sarcoma" as the name most suitable for designating neoplasia of mast cells.

A variety of names has been applied to the group of neoplastic expressions of the cells of the lymphoid series, such as "pseudoleukemia," "aleukemic leukemia," "lymphoblastoma," "lymphomatoid diseases" and "malignant lymphoma." The term "lymphosarcoma" most adequately encompasses this group whether the origin is local or diffuse in the lymphoid tissues and whether or not diagnostic cells are found in the

peripheral blood. Thus, the cancers of the cells of the lymphoid series, grouped under the basic name "lymphosarcoma," may be divided into stem cell (reticulum cell, reticuloendothelial cell), lymphoblastic, lymphocytic and giant follicular or nodular types. The findings of only lymphoblastic and lymphocytic types of lymphosarcoma in the 16 cases described does not necessarily indicate that other types will not be observed with the examination of more cases. That lymphosarcoma is as cancerous as any neoplasm, if not the most cancerous of all neoplasms, found in the dog was proved by a total clinical course of only two to seven months, during which the affected animals showed inappetence, loss of weight and strength, respiratory distress, anemia and terminal exhaustion.

The three basic cells widespread throughout the connective tissues of the body are the fibroblast, the mast cell and the histiocyte. Even though the cells of the lymphoid series have a wide distribution, the skin is not a primary site of their origin. When found in the skin, they are cells of inflammatory or neoplastic character which circulate in the peripheral blood and which migrate from the blood vessels into the cutaneous tissues. Therefore, when the skin, sometimes that of the genitalia, of young dogs acquires a small neoplasm that develops rapidly (two to six months), is clinically benign and histologically active (such a neoplasm as has been called "benign round cell sarcoma," "benign lymphosarcoma," "transmissible lymphosarcoma" and "extraveneal lymphosarcoma"), doubt arises as to whether the neoplasm actually consists of cells of the lymphoid series, for its clinical course is quite innocent, in striking contrast to that of the fatal lymphosarcoma. This noncancerous cutaneous, sometimes genital, neoplasm consists of cells with the distinctive features of neoplastic histiocytes and has been called "histiocyoma." Since the cells of the cutaneous tumors were identical with those observed on the genitalia, the evidence supports the idea that the much debated "venereal sarcoma" previously discussed²⁰ may be simply "histiocyoma." Indeed, other investigators¹⁴ have thought that the cutaneous and genital neoplasms they examined were identical histologically, although they used the name "transmissible lymphosarcoma."

SUMMARY

Mast cell sarcoma is a neoplasm characterized as follows: 1. It occurs in dogs usually 6 years of age or older. 2. Its incidence is greater in female dogs. 3. It shows a predilection for the Boston terrier, especially the female of the breed. 4. It has a tendency to involve the extremities (usually the hindlegs), the scrotum, the flanks, the vulva and the eyelids. 5. It reaches a size of 10 cc. or more in about 70 per cent of cases. 6. Its growth is rapid; it is poorly delineated from surrounding tissues; there is a strong tendency for it to recur after excision; frequently the surface is ulcerated; the cut section is

firm, lobulated, whorled, gray, pink-gray or white; there is multiplicity of growth in about 8 per cent of cases and involvement of regional lymph nodes in over 15 per cent of cases. 7. There is anaplasia of the constituent neoplastic mast cells as exemplified by size, number and ease of staining of specific granules, the ratio of nuclear size to cytoplasmic volume, the variation of the size and the number of nuclei, the multiplicity of nucleoli, the chromatin pattern and mitosis. 8. There is increased anaplasia in recurrent neoplasms.

Lymphosarcoma is a neoplasm which occurs usually in dogs 6 years of age or older; is perhaps more common in male and spayed female dogs; has a tendency to affect most often the Scotch terrier and the cocker spaniel; has a swiftly developing course and a fatal termination; always involves lymph nodes, often the spleen and the liver and less commonly other organs and tissues; attains large and symptom-producing size most frequently in lymph nodes; consists of lymphoblasts or of lymphocytes with characteristic cytologic features, and rarely gives rise to leukemic manifestations.

Histiocytoma is a neoplasm found predominantly in dogs 2 years of age or younger; is apparently more common in males; seems to favor the cocker spaniel; is usually located on the extremities, the face and the thorax, but seldom on the genitalia; is less than 5 cc. in volume in over 80 per cent of the cases; is acquired; grows rapidly, does not recur after excision and may regress spontaneously; is firm, white or gray and often ulcerated, and consists of neoplastic histiocytes with hazy borders, fairly abundant cytoplasm and nuclei of varying shape, which display homogeneous fine chromatin, small nucleoli and frequently mitotic figures.

Case Reports

FATAL VIRAL HEPATITIS COMPLICATED BY PHLEGMONOUS CECITIS AND ILEOCECAL INTUSSUSCEPTION

COMMANDER WILLIAM UMIKER (MC), U.S.N.
BETHESDA, MD.

ALTHOUGH intestinal complications of acute hepatitis are not uncommon, reports in the medical literature are scant. Bergstrand¹ described phlegmonous inflammation of the intestine complicating "yellow atrophy" of the liver. Pollack and Gerber² encountered suppurative inflammation of the intestine in 2 of 26 cases of "acute or subacute yellow atrophy" of the liver. Lucké and Mallory³ reported a 15 per cent incidence of phlegmonous intestinal inflammation in their series of 125 fatal cases of epidemic hepatitis.⁴ In each case there was associated ascites. Pollack and Gerber found this complication in other primary diseases of the liver, such as Laennec's cirrhosis or cholangiolitic cirrhosis, but in no disease without hepatic dysfunction. Mallory and Lucké found no instance of suppurative intestinal inflammation complicating fulminant epidemic hepatitis. The incidence appears highest in the cases of the subacute hepatic infections.

While the most striking intestinal complication is phlegmonous inflammation, there are others. Hemorrhages of varying size in the intestinal wall and mesentery, noninflammatory edema of the small and large intestine and changes in the lower third of the esophagus have been reported.⁴ The esophageal findings are similar to those described by Bartels as acute ulcerative esophagitis.⁵

I have been unable to find any report of intussusception complicating hepatitis. A case exhibiting this feature is herewith presented.

REPORT OF A CASE

A 60 year old white man was admitted to the United States Navy Hospital, Bethesda, Md., in a comatose state. His past history revealed that he had been

This article has been released for publication by the Division of Publications of the Bureau of Medicine and Surgery of the United States Navy. The opinions and views set forth are those of the writer and are not to be considered as reflecting the policies of the Navy Department.

1. Bergstrand, H.: Ueber die akute und chronische gelbe Leberatrophie, Leipzig, G. Thieme, 1930.

2. Pollack, A. D., and Gerber, I. E.: Arch. Path. **36**:608, 1943.

3. (a) Lucké, B.: Am. J. Path. **20**:471, 1944; (b) Mil. Surgeon **99**:89, 1946. (c) Mallory, T.: J. A. M. A. **134**:655, 1947.

4. Lucké, B., and Mallory, T.: Am. J. Path. **22**:867, 1946. Lucké.^{3a,b} Mallory.^{3c}

5. Bartels, E. C.: Arch. Path. **20**:369, 1935.

hospitalized earlier in the same year with the diagnosis of chronic peptic ulcer for which a partial gastrectomy was performed three months prior to his present admission. During this period of hospitalization he received a total of 900 cc. of pooled blood plasma intravenously (one hundred and twenty days before the onset of symptoms).

Following discharge, he was said to have enjoyed comparatively good health, except for mild epigastric distress. This increased in severity during the few weeks preceding his final entry. On the day prior to admission he complained that he "felt extremely ill." It was noted that his skin was jaundiced, and later that day he lapsed into coma. There was no history of ingestion of toxic substances or of diarrhea, chills or fever.

At the time of admission examination revealed a comatose man who appeared considerably older than his stated age. His temperature was 96 F.; pulse rate, 120; respiratory rate, 20; blood pressure, 90 systolic and 70 diastolic. The skin and the scleras were icteric. The heart and the lungs were within normal limits. The area of hepatic dulness to percussion was less than normal. No abdominal masses were palpable, and rectal examination revealed nothing of significance. The results of neurologic examination were likewise unremarkable.

Urinalysis gave negative results. The red blood cell count was 4,400,000, with hemoglobin 90 per cent. The white blood cell count was 9,200, with a normal differential count; the blood urea nitrogen was 17 mg. per hundred cubic centimeters; the carbon dioxide-combining power, 37 volumes per cent; the blood sugar, 31 mg. per hundred cubic centimeters (this rose to 250 mg. per hundred cubic centimeters after intravenous administration of 75 Gm. of dextrose and remained elevated). The icteric index was 94; the van den Bergh test for bilirubin showed 13.4 mg. per hundred cubic centimeters. The cerebrospinal fluid was shown to be normal by chemical, cytologic and bacteriologic tests. An electrocardiogram indicated that the heart's action was within normal limits.

The patient became spastic, with bilateral Babinski reflexes, increased deep reflexes and absence of abdominal reflexes. Ankle and knee clonus were elicited. Despite intravenous injection of calcium gluconate, parenteral administration of penicillin, nikethamide and oxygen and other supportive measures, the patient died within seven hours after admission.

Autopsy (thirteen hours after death).—The body was that of a poorly nourished, aged-appearing white man with definite jaundice of skin and scleras.

The serosal membranes showed petechial hemorrhages.

The liver weighed 870 Gm. and measured 17 by 22 by 5 cm. The capsule was wrinkled and the consistency decreased. The surface was smooth and light brown. The cut surface presented a mottled, reddish yellow appearance, suggesting somewhat the "nutmeg liver" of chronic passive congestion. The biliary tract and the gallbladder were not remarkable.

The peritoneal cavity contained 350 cc. of cloudy yellow fluid.

The entire gastrointestinal tract was edematous. The terminal 15 cm. of the ileum was telescoped into the cecum and the ascending colon. This was reduced with difficulty because of the extreme edema of the involved segments. The freed portion of the ileum exhibited edema and minimal fibrinopurulent exudate on the serosal surface. The cecum disclosed striking edema of all its layers, with the wall measuring 13 mm. in thickness. Despite hemorrhagic edema, the mucosa did not appear ulcerated. The mesentery and the mesocolon were slightly swollen but showed no evidence of infarction. Neither the portal venous system nor the mesenteric arteries were occluded.

The spleen weighed 100 Gm. and presented a normal color, structure and consistency.

The heart weighed 300 Gm. The coronary arteries showed calcified atherosclerotic plaques. The myocardium was dark brown and streaked with thin white strands. The lungs weighed 670 and 780 Gm., respectively, and were emphysematous, moderately edematous and congested. Their cut surfaces were brown to red, and crepitation was diminished. Slight subapical fibrosis was noted bilaterally.

The kidneys weighed 210 and 215 Gm., respectively. The capsule stripped with ease, revealing a finely granular surface with a few deep cortical scars. The cortex was narrowed, and the arteries were prominent. Corticomedullary differentiation was distinct. The cortex was pale, while the pyramids were deeply congested. Miscellaneous additional findings included a nodular thyroid gland, nodular hyperplasia of the prostate, scarring and deformity of the glans penis, bilateral testicular atrophy hypospadias, emaciation, multiple tattoo impregnations of the skin, generalized osteoporosis and edentulism. Examination of the brain was not permitted.

Microscopically, the liver showed a combination of severe necrosis and inflammation. There was extreme, irregular central necrosis with relatively few surviving hepatic cells. Those which persisted showed advanced cloudy swelling, degenerative fatty change and necrosis. Proliferation of bile ducts was especially pronounced. Foci of inflammatory cells were present throughout the portobiliary spaces. Some of these were neutrophilic granulocytes, but the majority were lymphocytes, plasmacytes and histiocytes. Many of the last contained brown pigment. Bile thrombi were numerous. No definite evidence of hepatic cell regeneration was detected. The gallbladder was not remarkable.

Sections through the cecum revealed extreme edema. There was an acute purulent exudate which extended through all the layers, with a fibrinopurulent exudate on the serosal surface. The veins and the capillaries were uniformly dilated and congested. A purulent endophlebitis was noted in the serosal layer. Sections through other portions of the large bowel as well as through the small intestine failed to disclose any changes other than edema.

The myocardium showed increased lipochrome pigment and diffuse fibrosis. Atherosclerosis was present in the coronary arteries as well as in the aorta.

The lung revealed acute exacerbation of chronic passive congestion. In addition, there were emphysema, patchy atelectasis and mucopurulent tracheobronchitis. Focal intra-alveolar hemorrhages were also present.

The kidneys disclosed evidence of biliary nephrosis as well as arterial and arteriolar sclerotic nephropathy. Subacute to chronic purulent cystitis was found. The prostate exhibited nodular glandular hyperplasia with chronic purulent prostatitis and prostatic urethritis. There was advanced atrophy of the testes. A mesenteric lymph node had undergone inflammatory hyperplasia.

The final primary pathologic diagnosis was: homologous serum hepatitis; phlegmonous cecitis, with ilocecal intussusception.

This case illustrates not only phlegmonous intestinal inflammation, similar to that described by the authors mentioned, but also complicating intussusception. The edematous cecum acted as intussusciens, and a portion of the terminal ileum, as intussusceptum. Although neither process was of long standing, it is probable that the inflammatory changes in the cecum antedated the intussusception. The difficulty of reduction and the absence of multiple similar intestinal invaginations seem to indicate that the process was not agonal.

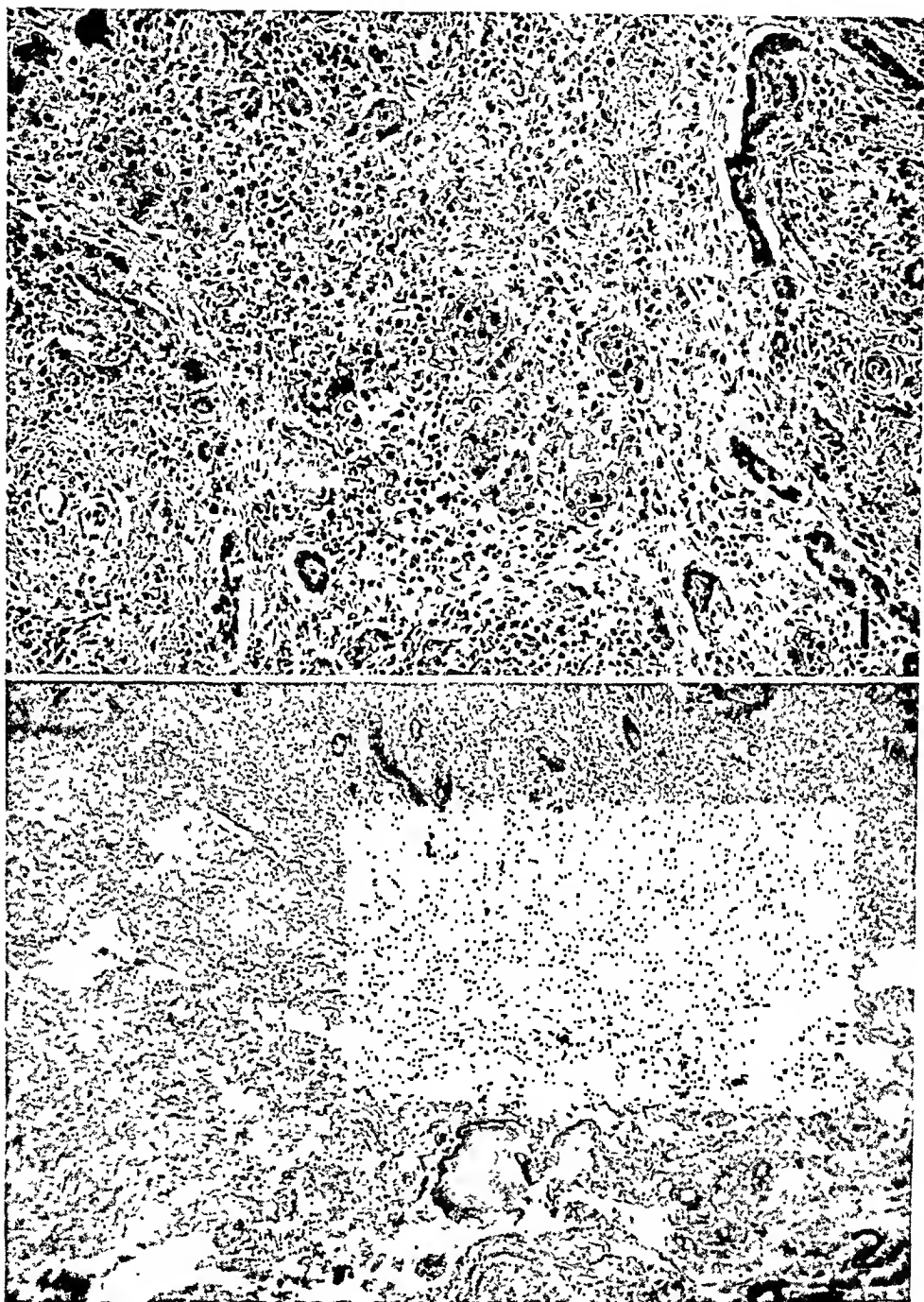


Fig. 1.—Homologous serum hepatitis. The liver shows severe necrosis and proliferation of bile capillaries at the periphery. A few remaining hepatic cells are seen. Note the bile thrombi in the canaliculi. Note scattered acute and chronic inflammatory cells.

Fig. 2.—Phlegmonous cecitis. This section through the cecum shows severe edema and leukocytic infiltration.

It is likely that the genesis of this phlegmonous inflammation is essentially similar to that of acute ulcerative esophagitis. In the latter the important factors are decreased resistance of tissues, due to impairment of the blood supply, plus the erosive action of hydrochloric acid. In the cecum, however, it is possible that the important factors are decreased resistance of tissues and a bacterially contaminated organ

Laboratory Methods and Technical Notes

METHOD FOR BIOPSY OF BONE MARROW OF EXPERIMENTAL ANIMALS

H. B. RITTER, B.A.

AND

J. J. OLESON, Ph.D.

PEARL RIVER, N. Y.

A TECHNIC to obtain pieces of intact marrow of sufficient size for histologic examination is as useful in experimental studies of animals as in clinical studies of anemias. Such a technic is of value also in obtaining complete hematologic data on the marrow while an experiment is in progress or prior to treatment of an experimental anemia.

The true status of the cells of the marrow cannot be shown if the death of the living tissue precedes fixation by more than a very few minutes. The problem of the study of marrow lies in the fact that the desired cells are enclosed within a heavily calcified structure which must be penetrated by the fixative as quickly as possible. The usual method of handling sections of marrow includes use of a decalcifying agent that will penetrate the bone along with the fixative. In some instances an adequate degree of fixation may be a matter of weeks, with consequent loss of staining power, due to postmortem changes in the marrow cells.

Mayer and Ruzicka¹ devised an ingenious method of exposing the marrow of a bone of a rat or a guinea pig within five minutes after the bone had been removed from the dead animal. The principles of their technic are herein applied to biopsies of the marrow of the bones of living animals.

The technic has been used on the femur of the rat for the most part. Biopsies have been made successfully on rats as young as 7 days of age. Young chicks, guinea pigs and rabbits have been used on occasion.

No aseptic precautions have been taken in the biopsies of rats and chicks, since it is well known that these animals are not exceedingly susceptible to infections. There have been no complicating infections at the sites of operation, although an occasional stitch abscess has been encountered. These have involved only the surface and subcutaneous layers of the skin in treated animals. Normal animals have shown no infections. Within the period of one week the bone is completely closed over with healthy granulation tissue. No loss of motion or of use of the bone has been seen.

The amount of marrow removed at biopsy can be controlled by the size of the incision made in the bone. There is little bleeding, and that only from the periosteal vessels. The marrow is removed intact

From the Lederle Laboratories Division, American Cyanamid Company.

1. Mayer, E., and Ruzicka, A. Q.: *Anat. Rec.* **93**:213, 1945.

and with little, if any, disturbance of cellular arrangement and topographic appearance, or contamination of peripheral blood. Small pieces of marrow can be obtained for smears simultaneously. Controlled experiments have shown that the biopsy has no effect on the marrow remaining in the intact bones.

The specimen is placed in the fixative immediately. In our hands the use of a neutralized, alcohol-formaldehyde fixative permits the use of Graham's peroxidase stain on the tissue *en bloc*.² This does not interfere with the use of any routine stain, and it allows the recognition of the granulocytic series of cells within the accepted limits of the peroxidase reaction.

The technic can be used with other animals if certain modifications, including asepsis and variation of the sizes of drills, are employed.

TECHNIC

Materials Required.—The following articles should be at hand:

Anesthetic ether and paper ether cone

1 pair of medium straight-blade scissors

2 pairs of straight fine forceps

1 piece of copper wire—6 inches long, $\frac{1}{8}$ inch in diameter (15 cm. long, 3 mm. in diameter)

Suture thread and needle or "Just Rite" skin clips

Dremel Moto-Tool Model 2³

Drills—steel cutter with elongated, serrated blade—catalog nos. S-111, 112, 113 and/or S-93, 94 and 95.

Method.—1. Light ether anesthesia is maintained in the rat by means of an improvised paper cone. The relaxed animal is placed on the table on its back with its right side parallel to the edge of the table if a biopsy of the right femur is to be made. The complete operation can be done by one person when the rat, the chick or the guinea pig is used. Maintaining the anesthesia of a larger animal may require the assistance of a second person.

2. An incision is made in the skin parallel to the direction of the femur, the knife cutting laterally toward the median line. The incision need not be more than $\frac{1}{2}$ to $\frac{3}{4}$ inch (about 1 to 2 cm.) in length, since the skin will stretch considerably.

3. The fat, subcutaneous tissues and blood vessels are spread free from the skin with a pair of fine forceps. This allows greater flexibility of the opening. At this point it is necessary to place one hand under the body of the animal so that manipulation may be made for changes in angles at the following steps. The thumb and the forefinger can be used as an added anchor for the dissected tissues when the drill is used in step 6.

4. The muscles over the femur are thus exposed. The closed blades of the forceps are inserted into the area of joining on the distal end of the femur. The forceps are then pushed along the line of cleavage, thus separating the two muscles and exposing the femur. By gripping the bone between the open blades of the forceps, one can strip the muscles clean from the femur by means of the

2. Ritter, H. B., and Oleson, J. J.: Arch. Path. 43:330, 1947.

3. This is supplied by the Dremel Manufacturing Company, Fourteenth and Clark Streets, Racine, Wis.

forceps. Care must be taken to free the muscles from the distal end of the knee joint to insure flexibility of the femur for the next step.

5. One blade of the fine forceps is inserted under the distal end of the femur as close to the joint as possible and is pushed between the muscle and the bone to the other side; thus the bone is raised out of the tissues. A piece of heavy copper wire is inserted in the same manner at the proximal end of the femur. This serves to lift the bone up and to hold the tissues down and back out of the way. The femoral artery and vein run parallel to the bone at this point but can be held with the wire if the binding tissues are loosened. The femur is scraped lightly to clean away the remnants of tissue with the blades of the forceps.

6. Starting at the distal end close to the joint and extending along the shaft of the femur for the desired distance, the drilling of the bone is carried on evenly along the length of the anterior surface so that no one section of marrow is exposed before any other. The size of the drill to be used in the moto-tool® depends on the size of the bone and its hardness. The length of the opening depends on the amount of marrow desired. The heat of the drill does not penetrate into the bone shaft if the drilling is done quickly. With practice one can expose the endosteum without puncturing through to the marrow. Bleeding at this point can be controlled by putting pressure on a piece of cotton held to the area.

7. The endosteum is lifted away with the fine forceps, exposing the marrow. With blades closed, the forceps is inserted vertically at the shaft end of the opening. The marrow is then scooped out by tipping the forceps toward the knee joint.

8. The marrow removed is placed directly in the fixative by shaking the forceps in the bottle of fixative. Further pieces of marrow close to the joint or at the end of the shaft opening may be obtained for smears.

9. The muscles are tucked back into place after the bone levers have been removed. The fat and subcutaneous tissues are shifted into approximately their former position. The skin is closed with interrupted sutures or skin clips. At this point the animal is usually out of the ether and will begin to walk around immediately. The clips may be removed in a few days.

Samples of marrow can be obtained from living animals by this biopsy technic. The quantity of marrow obtained permits a histologic study of marrow at any point in an experiment. The biopsy material can be used as a control for changes following further treatment of the same animal. With the larger animals, the procedure suggests that repeated biopsies may be made at stated intervals. With the smaller animals, one intact bone is available for terminal study.

SUMMARY

A technic is offered for biopsy of the marrow of the living young animal. The rat, the guinea pig, the rabbit and the chicken have been used.

Notes and News

Appointments.—R. Schrek, chief of the tumor research unit of the Veterans Administration Hospital at Hines, Ill., has accepted appointment as assistant professor of pathology in the Northwestern University Medical School, Chicago.

J. L. Arteta was recently appointed professor of histology and pathologic anatomy of the Faculty of Medicine of Santiago de Compostela, Spain. He is of the school of Cajal.

Harry L. Clark, after having been associated with Wayne University, Detroit, nearly forty years, is now emeritus professor of bacteriology and clinical pathology. He joined the faculty in 1919 and has held the rank of professor of bacteriology and clinical pathology since 1922.

J. M. Ravid, formerly pathologist at the Israel Zion Hospital, Brooklyn, has been appointed pathologist to St. Clare's Hospital, New York.

Edward A. Gall, assistant professor in the University of Cincinnati College of Medicine, has been appointed Mary M. Emery professor of pathology and head of the department, also director of pathology in the Cincinnati General Hospital. He succeeds the late R. S. Austin.

Alan R. Moritz has been appointed professor of pathology in the Western Reserve University School of Medicine and director of pathology in the University Hospitals of Cleveland. At present Dr. Moritz is professor of legal medicine at Harvard Medical School and pathologist-in-chief at Peter Bent Brigham Hospital, Boston. In his new post he will succeed Howard T. Karsner, retired, who has been appointed Medical Adviser to the Surgeon General, United States Navy.

Cushman D. Haagensen, coordinator of cancer teaching at Columbia University College of Physicians and Surgeons, has been named director of the cancer institute of the Columbia-Presbyterian Medical Center. Dr. Haagensen is also assistant attending surgeon and assistant surgical pathologist at the Presbyterian Hospital, New York.

New Laboratories.—The Public Health Research Institute of the City of New York has completed preliminary work toward setting up a laboratory for determining the Rh factor in human blood. Another laboratory recently developed performs virus diagnosis for physicians free of charge. Both laboratories are operated by the Bureau of Laboratories of the Department of Health.

A virus laboratory is to be set up by the University of Buffalo at Buffalo General Hospital. To help with its organization, Gilbert Dalldorf, Albany, director of the Division of Laboratories and Research in the State Department of Health, has accepted appointment as visiting professor of pathology at the medical school.

Books Received

HEMATOLOGY. By Cyrus C. Sturgis, M.D., professor of internal medicine and chairman of the department of internal medicine of the University of Michigan Medical School, and director of the Thomas Henry Simpson Memorial Institute for Medical Research of the University of Michigan. Pp. 915, with 81 illustrations. Price \$12.50. Springfield, Ill.: Charles C Thomas, Publisher, 1948.

This book deals with the diseases of the blood in 21 substantial chapters. Technical methods are not described except that chapters 22 and 23 deal with sternal puncture and with transfusion, respectively. There are 9 colored plates illustrating the blood affected by various diseases; all hematic preparations were stained by Wright's method and magnified 960 times and are well described. Of the 71 black and white figures, many are charts, some of which are not easy to read on account of the small print. This is true also of some of the tables. The text summarizes present knowledge of the diseases of the blood with special reference to the historical development of such knowledge. Each chapter contains a section on the history of the disease in question. The bibliography contains 1,830 carefully selected items. Numbered in the text, the references are placed at the bottoms of the pages, and there is also an alphabetical list (50 pages) at the end of the book. In some cases German nouns in the references are not capitalized. The book contains a vast amount of reliable information about blood diseases and will be of great help to physicians and advanced students. There is a tendency to prolixity, which should be controlled in future editions.

COLLECTED PAPERS OF THE MAYO CLINIC AND THE MAYO FOUNDATION. Edited by Richard M. Hewitt, M.D.; A. B. Nevling, M.D.; John R. Miner, Sc.D.; James R. Eckman, Ph.D.; M. Katharine Smith, B.A.; Carl M. Gambill, M.D., M.P.H.; Elizabeth L. Skafte, B.A., and Florence Schmidt, B.S.E. Volume 39, 1947. Pp. 871, with 128 illustrations. Price, \$12. Philadelphia and London: W. B. Saunders Company, 1948.

NEUROSURGICAL PATHOLOGY. By I. Mark Scheinker, M.D., assistant professor of neuropathology and assistant professor of medicine (neurology), University of Cincinnati College of Medicine; neuropathologist and attending neurologist, Cincinnati General Hospital. Pp. 370, with 238 illustrations. Price \$8.75. Springfield, Ill.: Charles C Thomas, Publisher, 1948.

Almost half of the volume deals with injuries of the central nervous system. The first chapter considers cerebral swelling, a term introduced to denote the gross appearance of a brain with local or diffuse increase in bulk of one or both hemispheres. Cerebral swelling in this newly assigned meaning is characterized by tumefaction, edema and liquefaction, different stages of the same process. The alterations following injuries of the central nervous system are presented in a sequential manner, the text giving a moving rather than a static picture of the processes. Though preference is given to the author's own concepts, documented with appropriate case reports, references to historical and experimental data are not wanting. In the chapter dealing with neoplasms of the central nervous system it is emphasized that most of them may be recognized in preparations stained with hematoxylin and eosin. Special staining methods are of little help in the recognition of rare tumors which cannot be classified by routine examination. The modified classification of the neoplasms is simple and adequate for that purpose. The last two chapters deal with cerebral abscess and hydrocephalus. A bibliography with key references and an index conclude the volume. The illustrations are good and well reproduced, and the print is easy to read.

CYTOLOGIC CHANGES IN BRONCHOGENIC CARCINOMA FOLLOWING TREATMENT WITH NITROGEN MUSTARD (METHYL-BIS [β -CHLOROETHYL] AMINE)

EDWARD A. GAENSLER, M.D.

DONALD G. McKAY, M.D.

PAUL F. WARE, M.D.

AND

JOSEPH P. LYNCH, M.D.

BOSTON

THE first study of the effects of mustard gas on tumor tissue was made by Berenblum in 1929.¹ He attempted to accelerate the formation of tar-induced tumors in mice by increasing the local blood supply to the skin with mild irritants. Dichlorodiethylsulfide in 1:1,000 dilution was one of the irritants used. This chemical added to the carcinogenic coal tar, however, almost completely inhibited tumor formation. This protection was exerted even if the mustard was not added until the twelfth week of treatment. Slightly larger concentrations of the mustard gas solution could cause destruction of tumor tissue.² This characteristic appeared to be peculiar to mustard gas, since other skin irritants, with the exception of cantharidin, did not exert a protecting influence.³ Systemic action was demonstrated by the fact that glycolysis and respiration of slices of sarcoma in vitro were depressed in the presence of anticarcinogenic substances, such as mustard gas, dichlorodiethylsulfone and cantharidin.⁴

The nitrogen mustards were synthesized as potential offensive warfare agents and proved to have action similar to that of mustard gas, but, unlike the latter, they were water soluble and therefore more easily handled. Peters and collaborators⁵ at Oxford University, Dixon and Needham⁶ at Cambridge University and Gilman and Philips⁷ and

From the Thoracic Surgery Service and the Mallory Institute of Pathology of the Boston City Hospital.

1. Berenblum, I.: *J. Path. & Bact.* **32**:425, 1929.
2. Berenblum, I.: *J. Path. & Bact.* **34**:731, 1931.
3. Berenblum, I.: *J. Path. & Bact.* **40**:549, 1935.
4. Berenblum, I.; Kendal, L. P., and Orr, J. W.: *Biochem. J.* **30**:709, 1936.
5. Peters, R. A.: *Nature, London* **159**:149, 1947.
6. Dixon, M., and Needham, D. M.: *Nature, London* **158**:432, 1946.
7. Gilman, A., and Philips, F. S.: *Science* **103**:409, 1946.

authors in this country quoted by them have recently published reviews of the work done on these substances during the war years. The primary objective of these extensive and well organized studies, namely, the discovery of an effective antidote or "competitive fixative" in the treatment of mustard gas poisoning, was not as successful as, for example, the discovery of BAL (2,3-dimercaptopropanol) for the arsenical group of war gases. It was established early that after exposure to these mustard compounds a portion of the substance is almost instantly "fixed" to the skin,⁸ and the remainder is absorbed where it, too, is "fixed" within a few minutes.⁸ The great affinity of these chemicals for a wide variety of enzymes and tissue nuclear proteins⁹ makes it appear that introduction of "competitors" in the treatment of poisoning is doomed to failure unless treatment is instituted almost coincidentally with exposure.¹⁰ On the other hand, much knowledge was accumulated during this time concerning the selective action of these drugs on rapidly proliferating tissues and their nucleotoxic action, as well as their in vitro and in vivo inactivation of enzymes.

The affinity shown by the mustards for proliferating tissues, such as the hemopoietic and lymphatic systems and the intestinal epithelium, has never been shown by any other drug. This affinity, as well as Berenblum's work on its anticarcinogenic properties, suggested further study in this direction. Friedenwald and associates,¹¹ while investigating the effects of nitrogen mustards on the mammalian cornea, demonstrated almost complete inhibition of mitotic activity even when the substance was used in dilutions only one one hundredth of those which cause clinical signs of intoxication. Nucleotoxic action was demonstrated on the resting cells of the basal layers of the corneal epithelium by the occurrence of nuclear fragmentation. This effect on resting cells of rapidly proliferating tissues was further manifested by the fact that, although 50 per cent of bone marrow cells are usually in the resting stage, over 90 per cent of these cells disappeared within a short time after mustard poisoning. Such inhibition of mitosis and nucleotoxic action was demonstrated not only in the cornea and bone marrow but also in tissue cultures of a large variety of normal and neoplastic cells.¹² In growing embryos these processes appeared to take

8. Black, S., and Thomson, J. F.: *Proc. Soc. Exper. Biol. & Med.* **63**:460, 1946.

9. Herriott, R. M.; Anson, M. L., and Northrop, J. H.: *J. Gen. Physiol.* **30**: 185, 1946.

10. Collumbine, H.: *Nature, London* **159**:151, 1947.

11. Friedenwald, J. S.; Buschke, W., and Scholz, R. O., in Moulton, F. R.: *Approaches to Tumor Chemotherapy*, Monograph, American Association for the Advancement of Science, Lancaster, Pa., Science Printing Company, 1947.

12. Karnofsky, D. A.; Burchenal, J. H.; Ormsbee, R. A., and Cornman, I.: *Cancer Research* **7**:50, 1947.

place selectively in proliferating areas only.¹³ Nuclear fragmentation as well as karyolysis are described in great detail by Friedenwald and associates.¹¹ Here, as following roentgen irradiation, changes in osmotic pressure and permeability of cell membranes appear to play a major role.

Mustard gas derivatives were able to induce mutations in *Drosophila*¹⁴ and *Neurospora*,¹⁵ a property heretofore thought to be peculiar to ionizing radiation. In fact, so strikingly parallel were the observed actions of the mustards and radiation that it was possible to compute that 100 moles of one of the nitrogen mustards exhibited the activity of one roentgen ray ion or 50,000 photons of ultraviolet rays.¹¹

Selective cytotoxic and nucleotoxic action on neoplastic tissue was therefore expected. Karnofsky and co-workers¹² observed that a "take" of mouse leukemia was prevented if donor mice had been previously treated with nitrogen mustards. Of several mustard derivatives tested, the methyl-bis and the tris compounds proved to have a definite "cytotoxic dose" for these leukemias. Cornman and Ormsbee¹⁶ noted that in rollertube cultures the observable response to methyl-bis (β -chloroethyl) amine (hereafter referred to as HN2) was quantitatively the same for normal and cancerous tissue but that the response occurred at different dose levels. Recently Karnofsky and co-workers¹² were able to kill selectively sarcoma 180 growing on the chorioallantoic membrane of the chick embryo without affecting the embryo's growth.

Gilman and Philips⁷ were the first to treat human subjects for neoplasms. Some time later Jacobson and co-workers¹⁷ independently began to treat similarly the neoplastic disorders of the hemopoietic and lymphatic systems. Since then there have been numerous reports on the efficacy of the nitrogen mustards in the treatment of Hodgkin's disease, leukemia and allied disorders. Promising remissions of Hodgkin's disease have been reported by many clinicians. Good results have also been obtained in the treatment of slowly growing lymphosarcoma, chronic leukemia and erythremia. Unfavorable results are reported in acute leukemia, rapidly growing lymphosarcoma and melanocarcinoma.¹⁸ It is the consensus at the present time that nitrogen mustard

13. Bodenstein, D.: *Cancer Research* **7**:49, 1947.

14. Horowitz, N. H.; Houlahan, M. B.; Hungate, M. G., and Wright, B.: *Science* **104**:233, 1946.

15. Auerbach, C., and Robson, J. M.: *Nature, London* **158**:878, 1946.

16. Cornman, I., and Ormsbee, R.: *Federation Proc.* **6**:390, 1947.

17. Jacobson, L. O.; Spurr, C. L.; Smith, T. R., and Dick, G. F.: *M. Clin. North America* **31**:3, 1947.

18. (a) Alpert, L. K., and Peterson, S. S.: *Bull. U. S. Army M. Dept.* **7**:187, 1947. (b) Sherry, M.: *South. M. J.* **41**:118, 1948. (c) Rhoads, C. P.: *J. A. M. A.* **131**:656, 1946. (d) Taffel, M.: *Yale J. Biol. & Med.* **19**:971, 1947. (e) ApThomas, M. I. R., and Collumbine, H.: *Lancet* **1**:899, 1947. (f) Jacobson and others.¹⁷

therapy is indicated when the neoplasms just mentioned become refractory to roentgen radiation or the disease is too widely disseminated for effective irradiation of the involved regions. Promising results have also been reported in the treatment of Boeck's sarcoid,¹⁹ mycosis fungoides,²⁰ neuroblastoma, Ewing's tumor and rhabdomyosarcoma.²¹

Bronchogenic carcinoma has been treated with HN2 with favorable results,^{18c} although so far only 2 cases in which an anaplastic type was treated thus have been reported in the literature.²² In both patients the carcinoma was far advanced, both received roentgen ray treatment concomitantly, and in both results appeared to be promising.

Only a few isolated studies of cytologic changes of neoplastic tissue following mustard treatment in man have been reported. Osborne and associates^{20b} obtained biopsy specimens of previously involved areas of skin from 2 patients with mycosis fungoides eleven and thirty days after treatment, respectively. In both instances there was no involvement of the tissues and no studies of cytologic changes were possible. We have had similar experiences in a number of cases discussed in the following section of this paper. Alpert and Peterson^{18a} studied lymph nodes excised from 2 patients with Hodgkin's disease three days after completion of the HN2 course. He found necrosis of reticulum cells; eosinophilic granulocytes and Reed-Sternberg cells. Sherry^{18b} made routine pretreatment and post-treatment biopsies of marrow in cases of Hodgkin's disease, and found pyknosis of nuclei of lymphocytes as well as clumping of cells. No cytologic studies were reported on either of the 2 cases of HN2-treated bronchogenic tumors referred to in the foregoing paragraph.

MATERIALS AND METHODS²³

During the course of a study of a large series of patients with inoperable or metastatic bronchogenic carcinoma treated with HN2 it was noted that certain patients responded more readily to this type of therapy than did others. Furthermore, though the degree of response was frequently related to the degree of anaplasia as judged from the microscopic appearance of the tumor, this was by

19. Snider, G. E.: *South. M. J.* **41**:11, 1948.

20. (a) Philpott, O. S.; Woodburne, A. R., and Waldriff, G. A.: *J. A. M. A.* **135**:631, 1947. (b) Osborne, E. D.; Jordon, J. W.; Hoak, F. C., and Pschierer, F. J.: *J. A. M. A.* **135**:1123, 1947. (c) Taffel,^{18d}

21. Cowdry, E. V.: *J.A.M.A.* **135**:1067, 1947.

22. Karnofsky, D. A.; Craver, L. F.; Rhoads, C. P., and Abels, J. C., in Moulton, F. R.: *Approaches to Tumor Chemotherapy*, American Association for the Advancement of Science, Lancaster, Pa., Science Press Printing Company, 1947. Alpert and Peterson.^{18a}

23. The nitrogen mustard used in this study was supplied by Merck & Co., Inc., through the agency of Dr. D. A. Karnofsky, of the Committee on Growth, of the National Research Council.

no means always the case.²⁴ It was felt that cytologic studies of tumor tissue before and after treatment might aid in explaining these differences in response and aid in an understanding of the mode of action of this drug. This palliative treatment was instituted only in cases in which there was microscopic evidence of bronchogenic carcinoma but the patients were unsuitable for surgical treatment. The entire series with its clinical aspects is being presented elsewhere.²⁴

The primary difficulty with such an undertaking was the inability to get biopsy specimens at the proper times after treatment. In some cases the original biopsy specimen was obtained at exploratory thoracotomy and there was no possibility of obtaining further biopsy specimens after HN2 treatment. In others the patients were seen some time after pneumonectomy with hepatic, cerebral or osseous metastases not accessible for biopsy. In still others the patients' general condition precluded repeated bronchoscopic examinations and removal of specimens.

In 12 cases tissue was available during or after treatment. In 4 of these cases although pretreatment and post-treatment specimens were obtained, the latter showed no tumor tissue histologically in an area previously known to contain tumor. Two patients had extensive involvement of the trachea at the time of the first bronchoscopy. A biopsy specimen was obtained with ease from each. Six days after treatment in one patient and eight days after treatment in the other no tumor could be observed on bronchoscopy and specimens taken from the previously involved areas, which now grossly appeared fibrotic, showed no tumor cells on microscopic observation and had the appearance of normal bronchial mucosa. There was no evidence of fibrosis or necrosis. Two other patients were seen about one year after pneumonectomy, at which time they showed great enlargement of cervical and submental lymph nodes. Biopsy specimens, obtained without difficulty, showed metastatic carcinoma in both. Five days after onset of treatment in one patient and twelve days after onset of treatment in the other the enlargement of the lymph nodes had grossly disappeared. In 1 case a specimen obtained after prolonged exploration of the submandibular region revealed fat and striated muscle only and there was no evidence of tumor, fibrosis or necrosis. In the other case the surgeon was unable to find any tissue suggesting tumor, and biopsy of several fragments similarly showed no remnants or other evidence of tumor. Since it is inconceivable that there should be no microscopic evidence of tumor, necrotic tissue or fibrosis so short a time after treatment, it must be presumed that the neoplastic tissue had shrunk to such an extent that the surgeon was unable to obtain a specimen of it at the correct place. These cases will not be discussed further.

In 8 cases adequate biopsy specimens were obtained after onset of treatment. In 1 case treatment was started while the patient appeared to be in terminal coma. He died ten days after the onset of treatment, and the specimen was obtained at postmortem examination. Another patient was treated immediately after exploratory thoracotomy and died ten days after termination of the HN2 course of total empyema and massive sepsis. Again tissue was obtained at postmortem examination. The third patient had a chain of large inguinal lymph nodes from which biopsy specimens were obtained seven days after treatment and again seven days later, and further tissue was obtained at postmortem examinations two months later. The fourth patient was subjected to cavernostomy for a large carcinomatous abscess of an upper lobe of a lung because of a septic course and large amounts of foul sputum. The lesion was subsequently found

24. Lynch J. P.; Ware, P. F., and Gaensler, E. A.: To be published.

to be inoperable, and the patient received four courses of HN2 over the course of a year. His draining cavity offered a unique opportunity for biopsy, and specimens were taken every other day for ten days after the onset of treatment in each course. Further material was obtained at postmortem examination shortly after the fourth course of HN2. In the remaining 4 cases tumor tissue was obtained before and after treatment from cervical lymph nodes or from the primary tumor at the time of bronchoscopic examination.

EFFECTS OF HN2 TREATMENT OBSERVED IN TUMORS

Epidermoid Carcinoma (squamous cell carcinoma, grade 1).—Formation of large multinucleated cells and cells with multilobed nuclei and fragmentation and disintegration of nuclei and cytoplasm are the principle changes noted in well differentiated squamous cell or epidermoid carcinoma after treatment with HN2. An unexpected finding in this type of tumor was a slight but definite increase in the number of mitoses.

These changes are best demonstrated in 1 of our cases in which, because of the easy accessibility of the tumor at the edge of a cavernostomy wound, multiple biopsies were made on successive days after treatment. Tissue was obtained before treatment and two, five and six days after treatment. The tumor histologically was a typical well differentiated epidermoid carcinoma with pearl formation, numerous mitotic figures and nuclei which were either round or oval and fairly regular in size and contained a single large basophilic nucleolus (fig. 1A). Fifty mitoses were counted in an area approximately 0.5 cm. square. Two days after treatment was begun, no morphologic difference from the pretreatment biopsy specimen could be detected with the exception of a slight increase in the number of mitotic figures. Sixty were counted in an area similar in size to that of the pretreatment biopsy specimen. The fifth day specimen revealed more definite changes. The number of mitoses had increased to 69 in a similar field. Several multinucleated cells were seen. Many nuclei had enlarged, were irregular in shape and vesicular and contained large, irregular clumps of chromatin material rather than a single round nucleolus (fig. 1B). There was a distinct increase in the number of cells with irregular, hyperchromatic nuclei. In addition, the connective tissue stroma had become edematous, and the nuclei of the fibrocytes, particularly in regions immediately surrounding clumps of tumor cells, had enlarged and were rounded rather than elongated and spindle shaped. Numerous polymorphonuclear leukocytes were scattered throughout the edematous stroma. The sixth day specimen showed the most striking changes. The number of mitoses per 0.5 cm. square was 105, a twofold increase. Many of these mitotic figures were atypical, including some with multiple spindles and bizarre forms, with chromatin material scattered through the entire cytoplasm of giant-sized cells. A number of cells were in various stages of necrosis and disintegration. Some of these were shrunken cells with pyknotic nuclei and bright red cytoplasm. Others were large cells with a well preserved cell membrane but without nucleus and a mass of both basophilic and acidophilic debris filling the entire cell. Numerous spaces about the size of one or two cells appeared within the tumor tissue. Some of these appeared empty; others were filled with basophilic clumps and acidophilic granules. The connective tissue changes seen in the specimen of the preceding day were again noted.

In the second case of epidermoid carcinoma the post-treatment biopsy was made one week after treatment was started. There was a slight increase in the number of mitoses, and many multinucleated cells with vesicular nuclei containing large, irregular masses of chromatin.

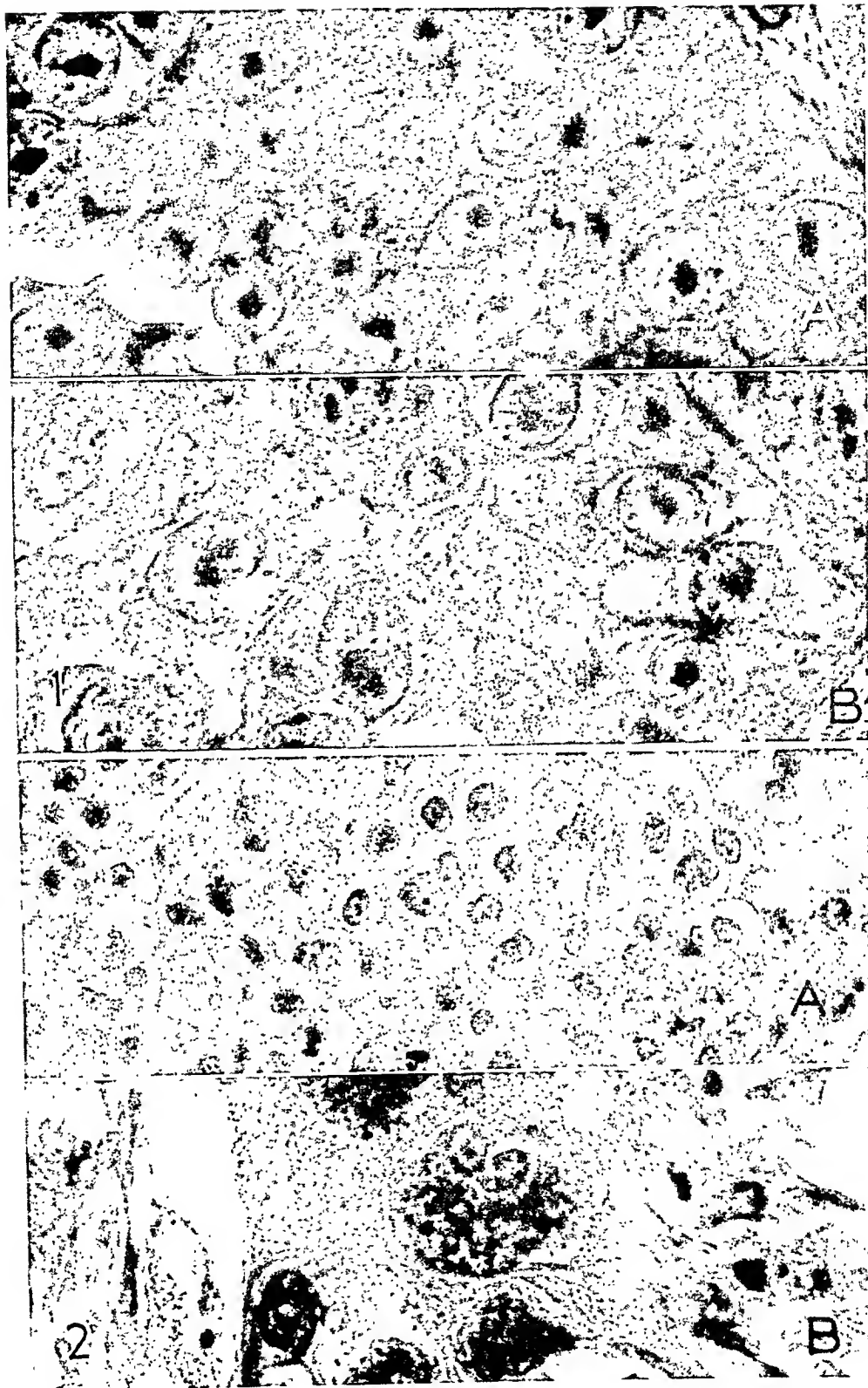


Fig. 1—Epidermoid carcinoma (case 1): *A*, before treatment. *B*, five days after onset of treatment. $\times 800$.

Fig. 2.—Epidermoid carcinoma (case 3): *A*, before treatment. *B*, ten days after onset of treatment. $\times 800$.

Two biopsy specimens were obtained in the third case. Here the most striking change was the formation of enormous tumor giant cells (fig. 2 *A* and *B*). The cells had enlarged to ten to twenty times their former size. Nuclear material occupied most of the cell and consisted of as many as twenty-five to thirty darkly staining pyknotic nuclei or nuclear fragments. These cells were not found in all parts of the tumor but were prominent in most areas. There was again a moderate increase in the number of mitoses.

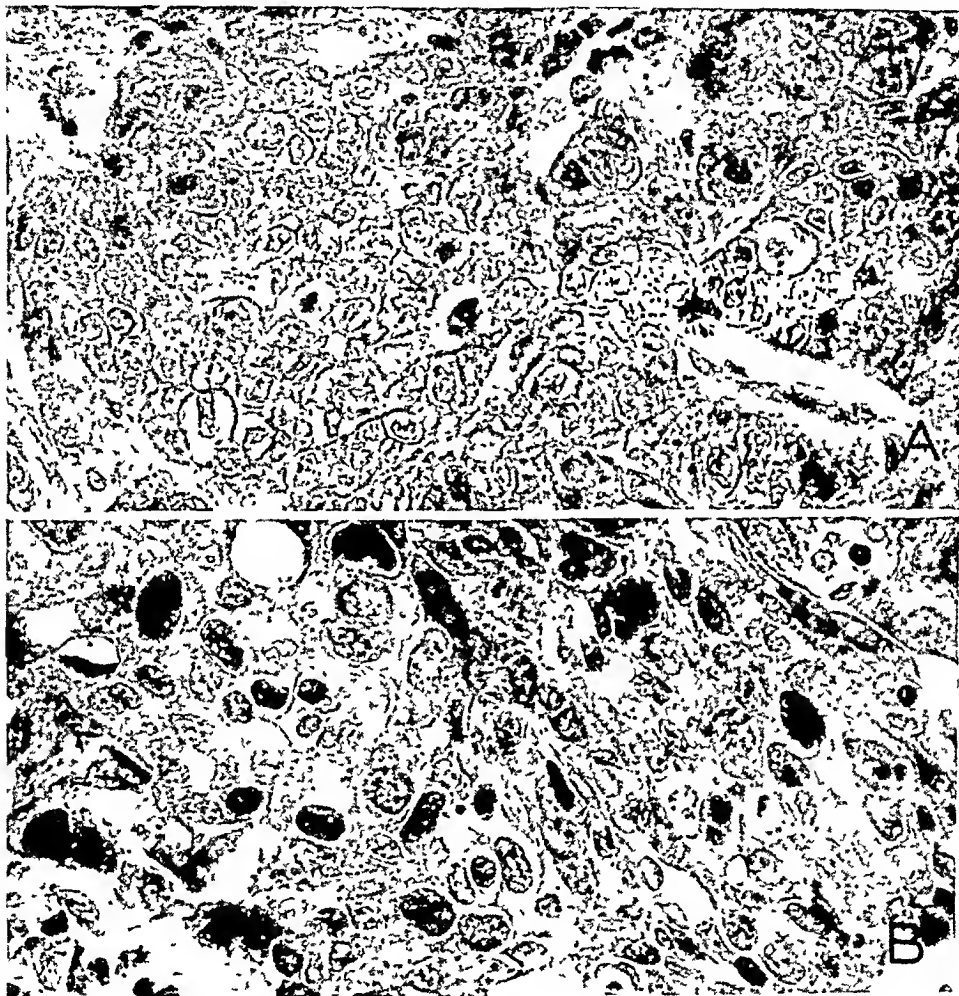


Fig. 3.—Squamous cell carcinoma: *A*, before treatment. *B*, ten days after onset of treatment. $\times 500$.

Squamous Cell Carcinoma (squamous cell carcinoma, grade 2).—Two cases of squamous cell carcinoma were available for study. Before treatment these tumors were made up of sheets of cells with nuclei of relatively uniform size containing prominent nucleoli (fig. 3 *A*). Many mitotic figures were present. A biopsy specimen taken five days after treatment showed numerous giant-sized nuclei which were vesicular and contained large, irregular masses of chromatin. A few multinucleated cells were present, which were not seen in the pretreatment specimen (fig. 3 *B*). There was a reduction of the number of mitoses.

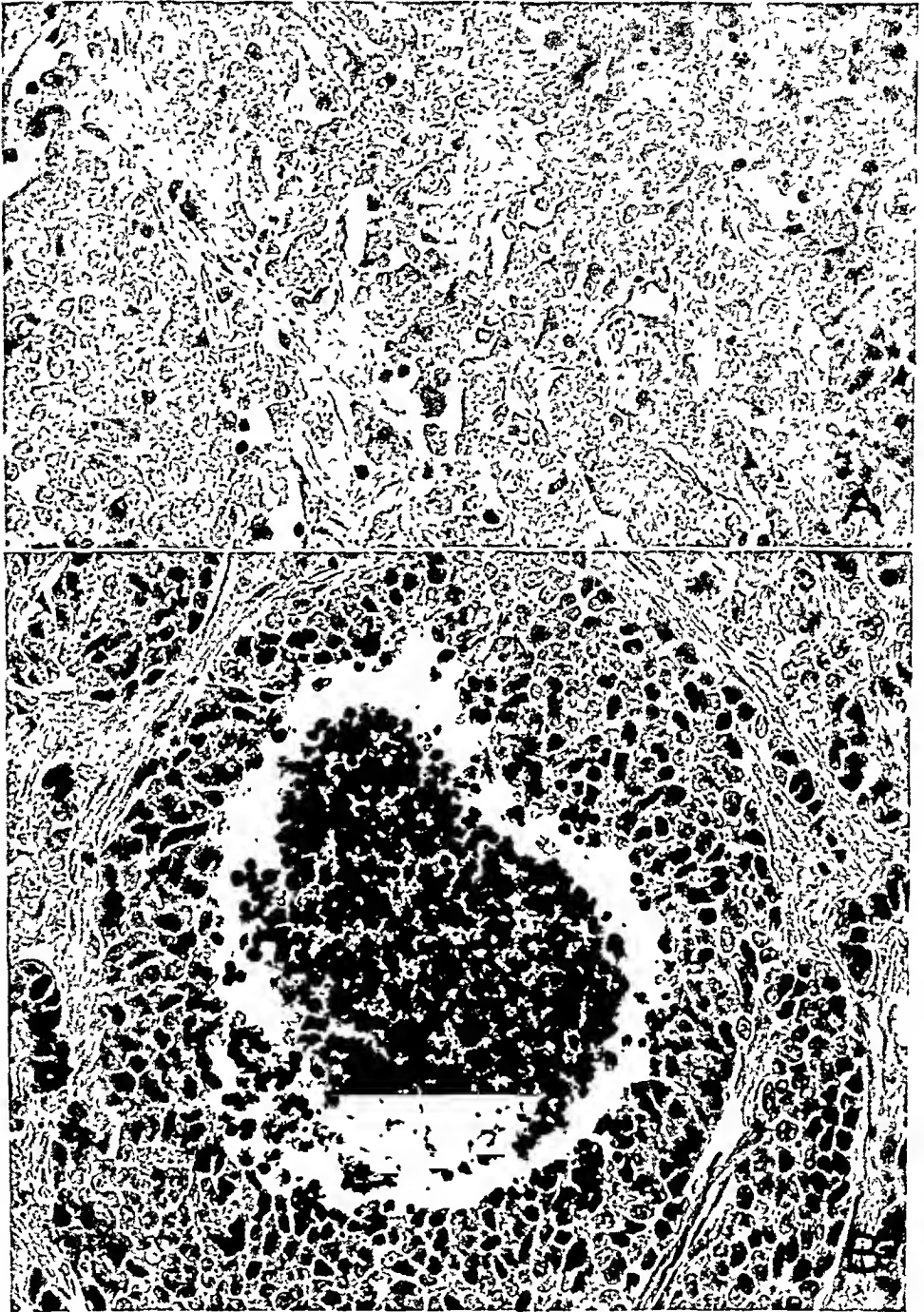


Fig. 4.—Undifferentiated carcinoma: *A*, before treatment. *B*, eight days after onset of treatment. $\times 400$.

Undifferentiated Carcinoma (oat cell carcinoma).—Although the gross reduction in size of the tumor masses of undifferentiated carcinoma was much greater than in the well differentiated tumors, the microscopic changes were not so striking. The most prominent change was necrosis of cells with reduction of the number of mitoses. Multinucleate forms and cells with irregular nuclei were not encountered in after-treatment sections. In 3 cases of undifferentiated carcinoma, biopsy specimens were taken five and eight days after the onset of treatment. In all cases, the tumor before treatment was composed of sheets and cords of small cells with small vesicular nuclei which did not vary greatly in size and shape. They had scant amounts of cytoplasm with indistinct cell membranes (fig. 4A). After treatment the tumor tissue showed a reduction of the number of mitoses. Foci of necrosis appeared in the centers of tumor areas. The nuclei became pyknotic and were fragmented. In some of these necrotic areas infiltrating polymorphonuclear leukocytes were noted (fig. 4B). A less obvious change was an increase of the number of cells having an irregular, hyperchromatic nucleus.

EFFECTS OF HN2 TREATMENT OBSERVED IN TISSUES NOT INVOLVED BY TUMOR

Autopsy material was available for study in 8 cases. Five of these were in our own series. In none of these cases was death directly due to HN2 treatment. Three of the patients died within ten days after conclusion of treatment, one of total empyema and overwhelming sepsis, another of terminal cachexia, and the third was admitted in coma and did not recover. The 2 patients remaining out of the 5 died about six weeks after treatment, one of multiple metastases involving almost every organ of the body, the other of tracheal obstruction.

The 3 patients not of our own series clearly died of the toxic effects of large doses of nitrogen mustard. Connerley and Sager gave us permission to review the material from 2 of these patients. The last was treated for mycosis fungoides and also died within ten days after the conclusion of treatment.

It was thus possible to observe not only the effects of toxic doses of the drug but the effects of doses within the therapeutic range and, in 2 instances, the picture following recovery from treatment.

It cannot be stated quantitatively what constitutes a "toxic" dose and what a "therapeutic" dose. It can be said, however, that no patient who received a total of 0.4 mg. per kilogram of body weight, the standard dose recommended by the Committee on Growth of the National Research Council, showed any toxic manifestations beyond brief leukopenia. All patients who died of toxic manifestations due to nitrogen mustard received 1.5 mg. per kilogram or more.²⁵ On the other hand, we have given up to 3 mg. per kilogram over the course of three weeks in some cases of our own series with survival.²⁴

Lymph Nodes.—Toxic doses of HN2 caused a marked alteration of the appearance of lymph nodes. The reticulum and the connective tissue were compressed, giving the nodes a fibrous appearance. This appearance may represent merely collapse of the node because of the loss of lymphocytes or a real increase of connective tissue (fig. 5A). Almost all lymphocytes had disappeared, and in their place were numerous plasmacytes and cells with acidophilic cytoplasm and large nuclei (fig. 5B). The latter had the general appearance of macrophages, but none were seen with phagocytosed debris within the cytoplasm.

In lower dosage HN2 did not produce effects as pronounced as those just described. Lymph follicles were present peripherally in the nodes and were

25. Connerley, H. L., and Sager, G. F.: Personal communications to the author.

made up of adult lymphocytes but were without germinal centers. The central regions of these nodes, however, had the same appearance as those receiving toxic doses.

The only nodes available from the patients who recovered from the effects of HN2 were filled with metastatic tumor. However, in one or two of these nodes numerous lymph follicles were seen and contained large germinal centers surrounded by masses of adult lymphocytes.

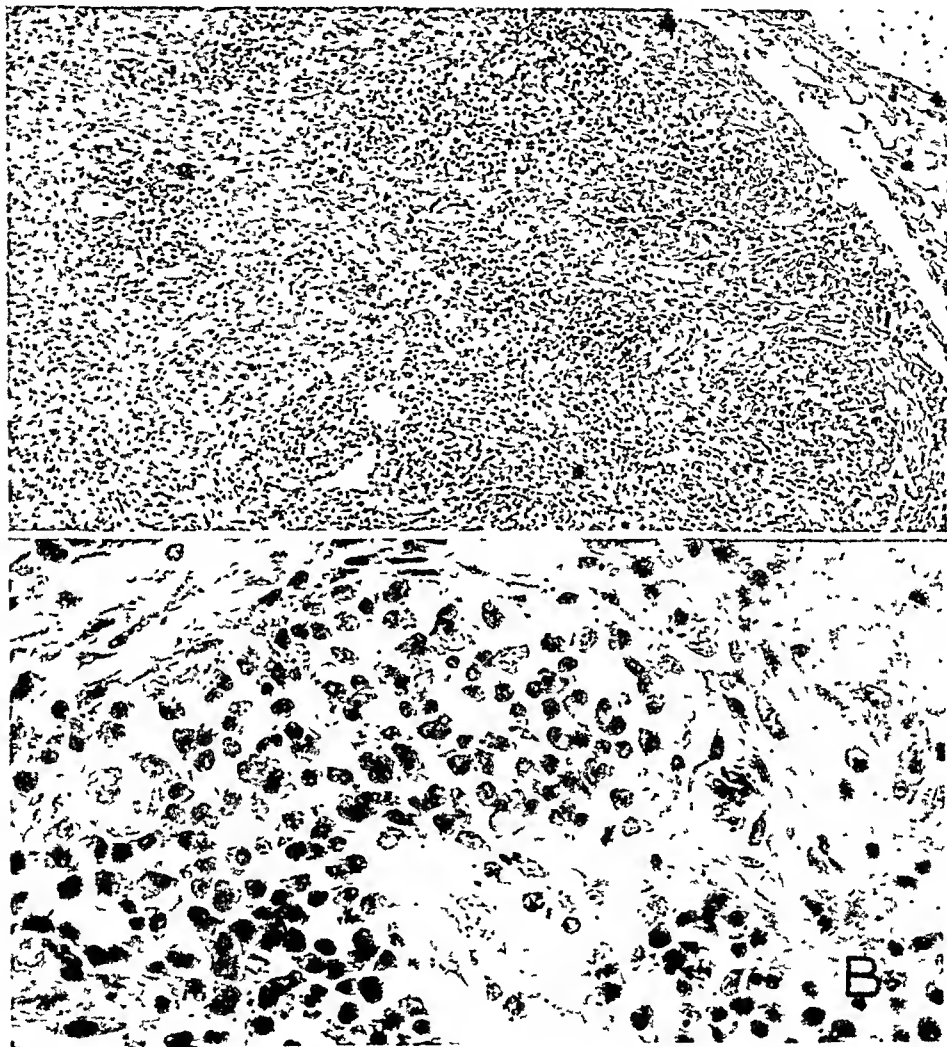


Fig. 5.—Lymph node: *A*, ten days after toxic dose of HN2; $\times 100$. *B*, ten days after toxic dose of HN2; $\times 500$.

Spleen.—A complete disappearance of malpighian corpuscles occurred following the administration of toxic doses of HN2 (fig. 6). A few lymphocytes could be found in the spleen. Numerous clumps of plasmacytes were seen; some of them were binucleate. In addition, there was a relatively large amount of deposited hemosiderin.

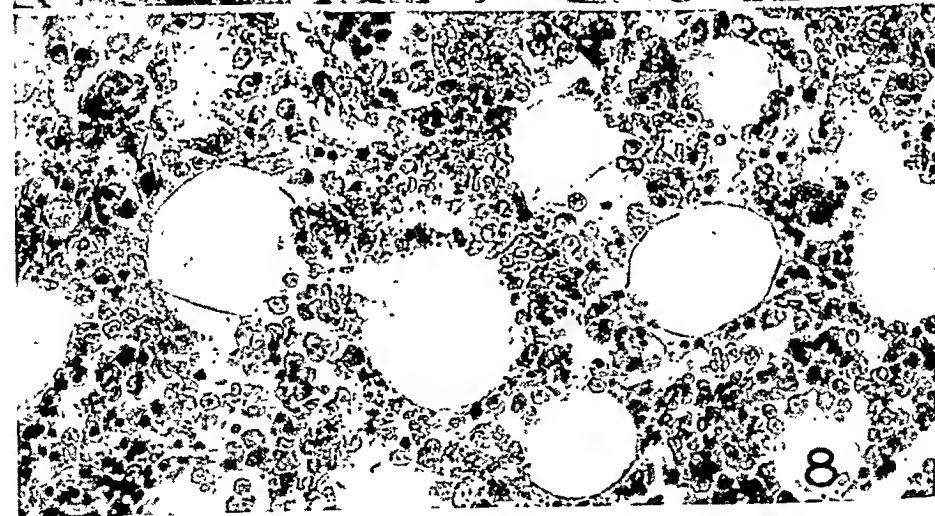
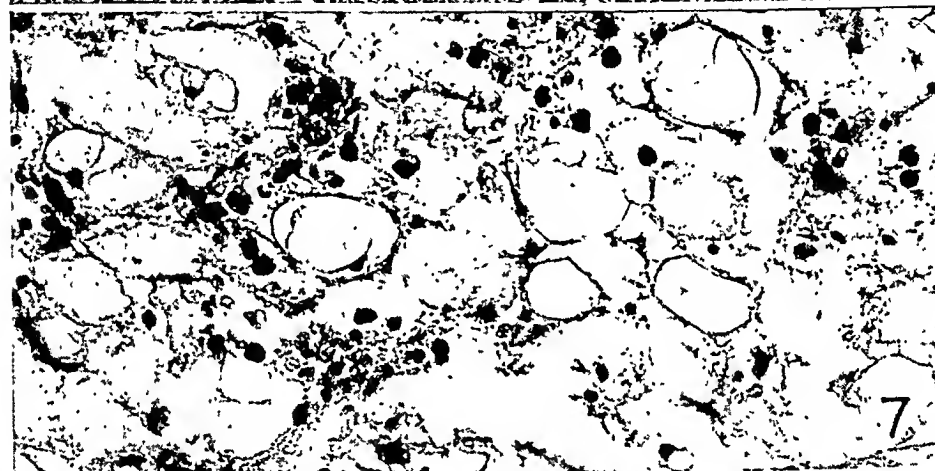
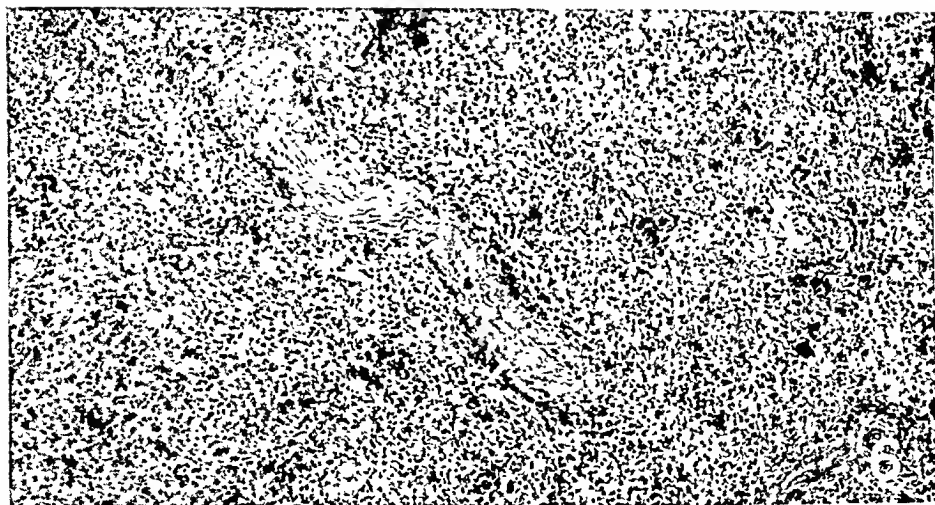


Fig. 6.—Spleen ten days after toxic dose of HN2; $\times 100$.

Fig. 7.—Bone marrow ten days after toxic dose of HN2; $\times 400$.

Fig. 8.—Bone marrow ten days after therapeutic dose of HN2; $\times 400$.

Malpighian corpuscles were present in the spleens of those patients receiving lower doses of HN2, and the only abnormal finding was an increased number of plasmacytes and large deposits of hemosiderin.

Bone Marrow.—A pancytopenia occurred in patients who died after toxic doses of HN2. The only cells remaining in the marrow of these patients were small collections of plasmacytes and a few unidentifiable cells similar to those seen in the lymph nodes. The sinusoids were filled with red blood cells and acidophilic granular debris, and there were numerous heavy deposits of hemosiderin. No myelocytes, prorubricytes, rubricytes, metarubricytes or megakaryocytes could be found (fig. 7). Many histiocytes contained red blood cells within their cytoplasm.

With smaller doses the changes occurring in the marrow were not so pronounced. In 1 case the sinusoids were congested and filled with red blood cells. There were numerous clumps of prorubricytes, rubricytes and metarubricytes, and a few megakaryocytes were present. However, this marrow contained almost no mature polymorphonuclear leukocytes and only a few clusters of myelocytes could be identified. The only change, therefore, was a diminution of the white cell series and the deposition of hemosiderin (fig. 8).

The marrow of the patients dying several weeks after treatment was extremely cellular, contained little fat, and had the appearance of a normal or a slightly hyperactive marrow.

Lungs.—Two of the 3 patients who died of HN2 toxicity had multiple small pulmonary hemorrhages and 1 had rather severe pulmonary edema. No pulmonary changes were noted in the patients receiving lower doses.

Testis.—Two of the 3 patients who received toxic doses had a marked change in the tubules of the testis. The only cells remaining in these tubules were the Sertoli cells. No germinal cells were seen. One of these patients was 21 years old, but the other was over 40, and therefore the effects on the testis were difficult to evaluate. No effect on the interstitial cells was noted.

Adrenal Glands.—No morphologic changes were detected in the adrenal glands of the patients who received therapeutic doses. However, in the cases of HN2 toxicity there was a complete disappearance of lipid from the cortex.

No morphologic changes were observed in intestine, kidney, liver, thyroid gland or pancreas.

COMMENT

On reviewing the sections of tumor, two facts stand out above all others: First it must be noted that every one of the after-treatment biopsy specimens, when examined by itself, shows a picture which is frequently found in tumors which have not been subjected to toxic substances or irradiation. Nuclear fragmentation, disintegration of cells and groups of cells, focal areas of necrosis and variations of granularity and of staining reaction are all seen frequently on examination of any group of tumors of this type. Even formation of large giant cells with enormous multilobed nuclei is not unusual. Secondly it is evident that when the sections are viewed in relation to the pretreatment specimen every one of them shows a definite change in the histologic appearance of the tumor. It is interesting that the most unquestionable and striking changes occurred in the highly differentiated type of tumor. Increase of the number of mitoses, giant cell formation and nuclear and cellular

disintegration were most noteworthy in this group. On the other hand, the least pronounced changes occurred in the undifferentiated cell type where areas of necrosis are so frequently found without previous chemotherapy or irradiation. Necrosis was accepted as a change due to HN2 treatment only because there was none in the pretreatment specimen and because this offered the only explanation for the decrease in size of involved lymph nodes with treatment. The only definite change here was a decrease of mitotic activity. The gross reduction of size of tumor as well as the favorable clinical response were most marked in the undifferentiated type.²⁴

It is possible that this seeming paradox can be explained by existing knowledge. Bodenstein¹³ has been able to show that cells of the embryo of *Amblystoma punctatum* show two types of reaction to HN2 exposure, a striking increase in size of well differentiated cells and a rapid disintegration in proliferating areas. Friedenwald and co-workers¹¹ similarly showed formation of very large cells in the epithelial layers of the cornea when mitoses were inhibited with repeated minute doses of HN2. The differentiating areas of epidermoid bronchogenic carcinoma show giant cell formation from which further tumor growth may occur, while the more rapidly proliferating areas show necrosis. Regaud²⁶ felt many years ago that epidermoid or other highly differentiated tumors developed in two directions, a further growth of neoplastic tissue cells, the *souche cellulaire*, and a collateral development, the *lignes laterales*. The latter type of development, he felt, leads to a cornified squamous layer and pearl formation and thus takes no part in a further invasive process and is ultimately self destructive. According to this, only the *souche cellulaire* is affected by radiation therapy. A parallel to HN2 effect might be drawn.

Clinical results can be correlated with these morphologic observations. Of 60 patients treated with HN2 for bronchogenic carcinoma, 54 per cent showed objective evidence of improvement. When the results are analyzed according to type of tumor, 83 per cent of undifferentiated tumors, 50 per cent of squamous cell tumors, 33 per cent of adenocarcinomas and 11 per cent of epidermoid carcinomas showed such objective evidence of improvement.²⁴

Of particular interest is the fact that the changes following HN2 treatment are practically identical with changes seen after ionizing irradiation. Roentgen ray changes in normal and neoplastic tissue have been amply investigated in the past. Approaches to the study of radiation effects have been made along lines similar to those along which the effects of exposure to alkyl amines are being investigated at the present time. Spear²⁷ has made an extensive review of the tissue culture

26. Regaud, C.: Radiophys. et radiotheran. 1:443, 1930.

27. Spear, F. G.: Brit. J. Radiol. 8:68, 1935.

approach. Warren²⁸ has studied the effects on living animals, and Stewart²⁹ has reviewed the cytologic changes occurring in human neoplasms after these have been exposed to roentgen radiation. Not only have the modes of study been similar but the results have been parallel. In both cases it is the nucleus which appears to suffer first and primarily. The "law of radiosensitivity" appears to apply to the alkyl amines. In both instances there is an almost instant chemical reaction within the cell, and in both there is a latent period before morphologic changes occur. Graef and co-workers³⁰ have recently published a study of the pathologic effects of lethal doses of HN2 as observed in animals. From their autopsy material showing involution of lymph nodes, thymus, spleen and bone marrow and necrosis of the intestinal tract they concluded that fatal roentgen irradiation produces effects in animals which are extraordinarily similar. They pointed out that even in such a small detail as the unusual HN2 resistance exhibited by chickens and pigeons there are similarities to the effect of ionizing radiation. A comparison of the two effects must be made on a superficial plane; the specific mode of action has as yet not been determined in either case.

The morphologic changes following toxic doses in normal tissue in these cases were in most respects similar to the changes found in animals.³⁰ We were unable to demonstrate changes in intestinal mucosa, since immediate autopsy was not possible in most cases and postmortem autolysis interfered with comparative study. One of the unusual autopsy observations might be mentioned at this point. In 2 cases death was due to overwhelming sepsis. The extent of the necrosis and the invasiveness of the infectious process were considered quite striking, particularly since both patients received full doses of penicillin for two weeks prior to death. This suggests that in addition to interfering with the cellular defense mechanism perhaps HN2 inhibits the antibody mechanism also. So-called "late deaths" of laboratory animals following mustard poisoning are frequently due to infection,³⁰ as are the late deaths following "total body" irradiation.²⁸ The growth of alpha hemolytic streptococci is inhibited when these cocci are grown in normal rat serum. Karnofsky and co-workers³¹ demonstrated that there is less inhibition if the streptococci are grown in serum of mustard-treated rats. The great affinity of the mustards for a wide variety of proteins and enzymes suggests that the antibody proteins might be affected also. It is suggested that nitrogen mustard treatment may be contraindicated in the

28. Warren, S. L.: *Arch. Path.* **34**:443, 562, 749, 917 and 1070, 1942.

29. Stewart, F. W.: *Arch. Surg.* **27**:979, 1933.

30. Graef, I.; Karnofsky, D. W.; Jager, V. B.; Krichesky, B., and Smith, H. W.: *Am. J. Path.* **24**:1, 1948.

31. Karnofsky, D. A.; Graef, I., and Smith, H. W.: *Am. J. Path.* **24**:275, 1948.

presence of severe infection not only because of possible leukopenia and agranulocytosis, which can usually be avoided with careful dosage and clinical observation, but also because of its effect on the antibody mechanism.

SUMMARY

Biopsy specimens of bronchogenic carcinoma were obtained and studied microscopically before, during and after nitrogen mustard treatment in 8 cases in which eleven courses of treatment were given.

Microscopic changes following treatment were distinct in the well differentiated tumors. They consisted chiefly in giant cell formation, nuclear fragmentation, increase of the number of mitoses and production of atypical mitoses. In contrast, in undifferentiated tumors a decrease of the number of mitoses and the appearance of large areas of necrosis were noted. The cytologic picture observed after treatment was as a whole similar to that found after exposure to ionizing radiation.

In 4 additional cases, the tumor had grossly disappeared from lymph nodes and the bronchial tree, and on section of previously involved areas there was no evidence of remaining tumor, fibrosis or necrosis.

Autopsy material was studied in 8 cases. Five of the patients had died of causes other than mustard treatment, while 3 received doses of the drug which proved to be fatal.

In toxic doses HN2 caused lymphocytes to disappear from lymph nodes, with condensation of connective tissue and accumulation of plasmacytes, loss of malpighian corpuscles of the spleen with deposition of hemosiderin and slight increase of the number of plasmacytes in the red pulp. In bone marrow there was complete absence of blood-forming cells, increase in number of plasmacytes and deposition of hemosiderin. The adrenal cortex showed disappearance of lipoid substance, and Sertoli cells were the only remaining structures of the testis.

In lower doses HN2 had no effect on the morphologic appearance of any organ except for diminution of myelopoietic tissue in the bone marrow with deposition of hemosiderin there and in the spleen.

EFFECT OF NITROGEN MUSTARD IN MYCOSIS FUNGOIDES

MATTHEW BLOCK, Ph.D., M.D.*

AND

JOHN C. MURPHY, M.D.

CHICAGO

FOR SEVERAL decades roentgen therapy has been the treatment of choice for mycosis fungoides. Because the cytotoxic effect of the nitrogen mustards rather closely resembled that of roentgen radiation,¹ these drugs have been used in treating tumors of the blood-forming organs² and more recently in the therapy of mycosis fungoides.³ It is our purpose to present detailed microscopic observations made before, during and after treatment with methyl-bis (β -chloroethyl) amine hydrochloride (a nitrogen mustard) in a case of mycosis fungoides.

* Senior Research Fellow, United States Public Health Service.

From the Sections of Hematology and Dermatology, Department of Medicine, University of Chicago.

This work was supported in part by the American Cancer Society, on recommendation of the Committee on Growth of the National Research Council.

1. (a) Block, M.: Unpublished material from the Metallurgy Laboratory of the Manhattan Engineering District. (b) Kindred, J. E.: *Arch. Path.* **43**:253, 1947.

2. Spurr, C. L.; Jacobson, L. O.; Smith, T. R., and Barron, E. S. G.: The Clinical Application of Methyl-Bis (B-Chloroethyl) Amine Hydrochloride to the Treatment of Lymphomas and Allied Dyscrasias, in Moulton, F. R.: *Approaches to Tumor Chemotherapy*, American Association for the Advancement of Science, Lancaster, Pa., Science Press Printing Company, 1947, p. 306. Goodman, L. S.; Wintrobe, M. M.; McLennan, M. T.; Dameshek, W.; Goodman, M. J., and Gilman, A.: Use of Methyl-Bis (B-Chloroethyl) Amine Hydrochloride and Tris (β -Chloroethyl) Amine Hydrochloride ("Nitrogen Mustards") in the Therapy of Hodgkin's Disease, Lymphosarcoma, Leukemia and Certain Allied and Miscellaneous Disorders, in Moulton, F. R.: *Approaches to Tumor Chemotherapy*, American Association for the Advancement of Science, Lancaster, Pa., Science Press Printing Company, 1947, p. 338. Karnofsky, D. A.; Burchenal, J. H.; Ormsbee, R. A.; Cornman, I., and Rhoads, C. P.: Experimental Observations on the Use of the Nitrogen Mustards in the Treatment of Neoplastic Disease, in Moulton, F. R.: *Approaches to Tumor Chemotherapy*, American Association for the Advancement of Science, Lancaster, Pa., Science Press Printing Company, 1947, p. 293.

3. (a) Post, C. F., and Lincoln, C. S.: *J. Invest. Dermat.* **10**:135, 1948. (b) Philpott, O. S.; Woodburne, A. R., and Waldriff, G. A.: *J. A. M. A.* **135**:631, 1947. (c) Henstell, H. H.; Tober, J. N., and Newman, B. A.: *Blood* **2**:654, 1947.

CLINICAL HISTORY⁴

A. P., a 26 year old married white woman, had been treated since the age of 13 for psoriasis, diagnosed clinically and histologically. At the age of 25 lesions developed which were characteristic of mycosis fungoides. They were treated with roentgen rays, to which they showed gradually decreasing responsiveness.

Between the sixth and twenty-ninth hospital days the patient received a total of 723 Gm. of para-aminobenzoic acid orally with no apparent change in the cutaneous or the laboratory findings, but with progressive deterioration of her general status.

Beginning on the thirtieth hospital day, nitrogen mustard (0.1 mg. per kilogram of body weight) was administered daily intravenously for four consecutive days. A dramatic clinical resolution of cutaneous lesions with melting away of tumors and healing of ulcers was apparent by the third day of treatment. This reached

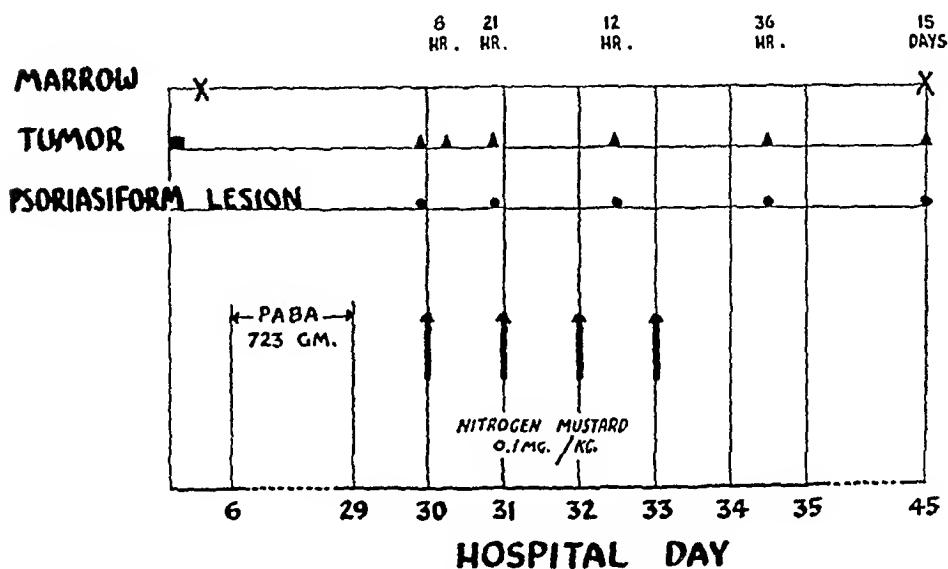


Fig. 1.—Time relation of biopsies and injection of nitrogen mustard (0.1 mg. per kilogram of body weight). PABA stands for para-aminobenzoic acid, administered to a total dose of 723 Gm.

a maximum on the eighth day, to continue more slowly thereafter. However, progressive leukopenia developed, beginning on the sixth day after the first injection of nitrogen mustard, and the white cell count reached a low of 375 one week later. On the seventh day after the onset of treatment the liver was tender and palpable. Scleral icterus, which appeared on the twelfth day, was followed by generalized intense jaundice. A hemorrhagic tendency developed on the thirteenth day, and thereafter the course was steadily downhill despite vigorous supportive measures, including multiple transfusions of whole blood and intravenous injection of toluidine blue. The patient died on the forty-fifth hospital day, fifteen days after the beginning of nitrogen mustard therapy.

MATERIALS AND METHODS

A tumor and the sternal marrow were submitted to biopsy prior to administration of para-aminobenzoic acid. Specimens of another tumor and a psoriasiform

4. The clinical history has been reported in detail elsewhere (Murphy, J. C., and Montgomery, H.: *J. Invest. Dermat.* 11:245, 1948).

lesion were taken for biopsy immediately before and at selected intervals after starting nitrogen mustard treatment. Tissue from the same two tumors and from the sternal marrow were also obtained at autopsy (fig. 1).

The tissues were fixed in neutral formaldehyde-Zenker solution for eight hours. They were dehydrated, embedded in pyroxylin (nitrocellulose) and cut serially at 6 microns on the sliding microtome. The slides were stained with hematoxylin-eosin-azure II.

HISTOPATHOLOGIC ASPECTS OF TUMOR-LIKE LESIONS

Prior to Administration of Nitrogen Mustard.—The major part of the surface was ulcerated. Where the epidermis was intact it contained broad, flat rete ridges extending deeply into the underlying tumor tissue. Mitoses were not unusual in number or in quality. A moderate infiltrate of inflammatory cells was present in the epidermis (fig. 2 A). The connective tissue-epidermal boundary was intact.

The papillary layer was replaced by a densely cellular mass of tissue consisting of numerous mycosis cells, plasmacytes, eosinophilic granulocytes, occasional neutrophilic granulocytes, some of which were degenerate, and mast cells. In the mycosis cells mitoses were numerous. Many mycosis cells had large angular blocks of chromatin in their nuclei, and the cytoplasm was stained very darkly at the periphery (fig. 2 B, a). Others had shortened, densely staining cytoplasmic processes with a pale paranuclear area. In the latter the nuclear chromatin was extremely heavy (fig. 2 B, b), similar to that seen in plasmacytes (fig. 2 C, c).

The reticular layer of the corium was essentially similar in cellular makeup. However, the nests of plasmacytes were more numerous and larger than in the papillary layer and the mycosis cells were present in large sheets.

There had been no significant change in the histopathologic appearance of the lesion following the administration of para-aminobenzoic acid. Minor changes, such as an increase in eosinophilic granulocytes, a more delicate appearance of the mycosis cells and fewer mitoses, were so slight that they were felt to be within the range of variation to be expected in comparing two different lesions from the same patient.

Eight Hours After the First Injection of Nitrogen Mustard.—Arborization of the acanthotic rete pegs had developed. The stratum granulosum had disappeared. The epithelial papillae were wider and more swollen than prior to treatment. An increase of the amount of debris or a cessation of mitosis was not demonstrable in the epidermis.

The most marked change was in the papillary layer. An intense edema of the ground substance separated the cells. The granules of some of the eosinophilic leukocytes were strewn about, and there was some karyorrhexis of the nuclei of these cells (fig. 2 C, b) as well as of the nuclei of the small lymphocytes (fig. 2 C, a). The mycosis cells were less bizarre in appearance, and in them mitoses were definitely decreased in number. An occasional plasmacyte was seen with clumping of nuclear chromatin, but in general degeneration of plasmacytes was less frequent than that of lymphocytes and eosinophilic granulocytes. The blood vessels were dilated and filled with a basophilic precipitate.

Similar but less intense changes were seen in the reticular layer.

Twenty-One Hours After the First Injection.—Acanthosis was less marked than in the previous biopsy specimen. The cellular infiltration and edema of the epidermis were decreased in amount.

The eosinophilic granulocytes in the corium, many of which were degenerate, were greatly increased in number. The plasmacytes were decreased. There was



Fig. 2.—*A*, tumor-like lesion as it appeared prior to injection of nitrogen mustard. ($\times 104$.)

B, same lesion showing a pleomorphic infiltrate in the papillary layer. Note mycosis cells, (*a*) with a heavy nucleus and (*b*) with a pale paranuclear area, and a plasmacyte (*c*). ($\times 980$.)

C, same lesion showing edema and cellular degeneration in the papillary layer eight hours after the first injection. Note (*a*) degenerate lymphocytes and (*b*) degenerate eosinophilic granulocyte. ($\times 1,160$.)

little edema. Degenerating cells were rare. The mycosis cells had not changed further. Except for the eosinophils, the total cellularity of the infiltrate, especially in the papillary layer, was decreased.

Twelve Hours After the Third Injection.—The epidermis was still acanthotic and without a granular layer. There was no further increase in the number of degenerate nuclei.

The cellular infiltrate of the corium was still further decreased, except for the presence of numerous small and medium-sized lymphocytes (fig. 3A). The mycosis cells had not changed. The plasmacytes especially were decreased in number. In mycosis cells mitosis had ceased entirely. In the reticular layer of the corium most of the previously diffuse infiltrate was now perivascular, and one could see for the first time the feltwork of collagenous fibers between the vessels.

Thirty-Six Hours After the Fourth Injection.—The involution described in the foregoing section had progressed further, and there had also been a decrease in the number of small and medium-sized lymphocytes.

Fifteen Days After the Initial Injection (Autopsy).—Acanthosis and arborization of the rete mucosum were greatly reduced (fig. 3B). The stratum granulosum was still absent.

The infiltrate had almost completely disappeared from the papillary layer, but occasional mycosis cells were still present. In the reticular layer the infiltrate was still quite dense and numerous mycosis cells were present, in some places in sheets. The lesion was not as pleomorphic as when seen originally, because of a relatively greater decrease in the number of free cells (eosinophilic granulocytes, plasmacytes, lymphocytes and neutrophilic granulocytes) than of fixed cells (mycosis cells). Most of the remaining infiltrate was perivascular. In mycosis cells mitoses were infrequent. Nevertheless, the lesion was still clearly recognizable as mycosis fungoides in a somewhat more acellular state as regards the free cells than existed prior to treatment (fig. 3B).

HISTOPATHOLOGIC ASPECTS OF THE PSORIASIFORM LESION

Prior to Administration of Nitrogen Mustard.—The epidermis was moderately acanthotic and slightly invaded by lymphocytes (fig. 3C).

The papillae were thicker than usual. The ascending and descending capillary loops were surrounded by small lymphocytes (fig. 3C). An occasional mycosis cell was demonstrable, a few of them in mitosis. The fibroblasts were not increased in number, but their nuclei were a little swollen, and the cytoplasmic processes were thicker and more heavily stained than usual. A few macrophages with phagocytosed iron were seen.

The reticular layer of the corium was normal except for a perivascular lymphocytic infiltration about the subpapillary plexus and about the vessels connecting this plexus to the deep cutaneous plexus.

Twenty-One Hours After the First Injection.—There was an increase in acanthosis and arborization of the rete mucosum. The number of lymphocytes was increased, although this was counterbalanced by an increase in degenerating lymphocytes. Mitosis had ceased entirely. Although there was no hemorrhage, the number of macrophages with phagocytosed iron granules was increased.

Twelve Hours After the Third Injection.—Parakeratosis and acanthosis were still further increased. The number of lymphocytes had not changed. Mast cells were increased in number. There was no increase of degeneration. Mitoses were completely absent.



Fig. 3.—*A*, same lesion as in figure 2 *A*, showing all cells except lymphocytes decreased in number twelve hours after the third injection. ($\times 123$.)

B, same lesion as in figure 2 *A*, showing decreased acanthosis and decreased cellular infiltrate, especially in the papillary layer, at autopsy. ($\times 123$.)

C, flat lesion, prior to injection of nitrogen mustard, showing perivascular infiltrate. ($\times 245$.)

D, same lesion as in *C*, showing increased arborization and only slight decrease of infiltrate at autopsy. ($\times 245$.)

E, same lesion as in *C*, showing (*a*) increase in mast cells and (*b*) iron-containing macrophages at autopsy. ($\times 1,050$.)

Thirty-Six Hours After the Fourth Injection.—The acanthosis and parakeratosis were a little decreased. There was less lymphocytic infiltration, and what was present was clearly perivascular. Mitoses were present but decreased in number.

Fifteen Days After Initial Injection (Autopsy).—An abnormal amount of acanthosis was still demonstrable (fig. 3D). The amount of infiltrate was but slightly decreased as compared with that observed prior to treatment (compare fig. 3C and D). Mast cells (fig. 3E, a) and iron-containing macrophages (fig. 3E, b) were more numerous than before. There were numerous hemorrhages about the subpapillary plexus and about the vessels in the superficial portion of the reticular layer of the corium.

HISTOPATHOLOGIC CHANGES OBSERVED IN OTHER ORGANS

The pretreatment marrow was rather hypercellular. There was myelocytic hyperplasia with a mild left shift and an increase in cells of the plasmacytic series. On smear the megakaryocytes and the thrombocytes were normal.

At autopsy the marrow was atrophic—about one-fourth as cellular as it was prior to treatment. Most of the hypocellularity was due to the disappearance of granulopoietic and erythropoietic cells as well as of megakaryocytes. As a result, cells of the plasmacytic series and reticular cells were much more prominent. A great deal of the marrow was occupied by a gelatinous precipitate and dilated vessels.

At autopsy all the viscera, as well as the skin, were the site of numerous hemorrhages. In the liver many of the parenchymatous cells were necrotic, perhaps as a result of pressure from the numerous extravasated red cells. There was no evidence of lymphoma in any of the internal organs.

COMMENT

The effect of nitrogen mustards on tissues is similar to that of ionizing radiations. In the normal animal given median lethal to lethal doses of nitrogen mustard the successive changes are as follows: First there are cessation of mitosis and degeneration of susceptible cells (small lymphocytes, myelocytes, erythroblasts and megakaryocytes). The major part of the degeneration of susceptible cells occurs only in the hemopoietic tissues, within eighteen hours after injection, not in the peripheral blood. The outstretched fixed cells (fibroblasts, macrophages and reticular cells) as well as plasmacytes are extremely resistant to the destructive action of the mustards. The second phase is phagocytosis of debris. The third, or atrophic, phase lasts several days and corresponds to a period of aplasia of the susceptible cells and reduction of the number of mitoses. Regeneration ensues as a result of heteroplastic formation of the susceptible free cells from the reticular cells and homoplastically by mitotic proliferation of the few residual susceptible cells.¹ There is no evidence to support the thesis that the nitrogen mustards exert their effect primarily on immature or rapidly dividing cells.

Henstell and co-workers,^{3c} after serial observations on well controlled biopsy material, described a decrease in density of the infiltrate in

mycosis fungoides. They concluded that the drug exerts its effect primarily on "reticulo-endothelial cells." In their illustrations and descriptions, degeneration of such cells is not apparent. In any case it is rather doubtful that the mycosis cell belongs to the "reticulo-endothelial system" as originally interpreted by Aschoff.⁵

In general, our results confirm those of Henstell and co-workers^{8c} so far as the decrease in the infiltrate is concerned. However, in our material there was little evidence of any marked effect on the mycosis cells themselves except as regards the cessation of mitosis. There was always some degeneration in the mycosis cells prior to treatment, and there was no significant increase of this degeneration following treatment. Judging from the experimental work demonstrating the resistance of fixed cells, such as fibroblasts and reticular cells, to nitrogen mustard,⁶ one would hardly expect any marked destructive effect on the mycosis cells, which also are fixed cells. Inhibition of mitosis, coupled with spontaneous degeneration, causes a decrease in the number of mycosis cells. However, it is difficult to determine whether the combination of these two factors alone adequately accounts for the decrease in number of mycosis cells.

The histopathologic effects of nitrogen mustard in Hodgkin's disease and lymphosarcoma, which are related to mycosis fungoides, have been studied.^{6a} Parallel with our experience, in those diseases it was impossible to account for the decrease in size of the tumorous organs by the amount of cellular degeneration seen. As a result of numerous follow-up biopsies, it was emphasized that nitrogen mustard could destroy only those cells in tumors which it also destroys in normal tissue; in addition, inhibition of mitosis was described. At no time (again in agreement with the results of our study) was it possible to demonstrate a change in the basic structural pattern of any lesion at biopsy.^{6a}

Although our patient died before an exacerbation occurred, from other reports on the clinical course of mycosis fungoides following injection of nitrogen mustard⁸ there is little doubt that such an exacerbation, manifested by a proliferation of the residual mycosis cells and a reappearance of the free cells, would have occurred, in a manner analogous to that seen in Hodgkin's disease.^{6a}

Because of the similarity of the histopathologic effects of nitrogen mustard and roentgen radiation, it is interesting to compare the effects of the two on the lesions of mycosis fungoides. Unfortunately, there are few controlled observations of the histopathologic effects of the

5. Aschoff, L.: *Ergebn. d. inn. Med. u. Kinderh.* **26**:1, 1924.

6. (a) Block, M.; Spurr, C. L.; Jacobson, L. O., and Smith, T. R.: *Am. J. Clin. Path.* **18**:671, 1948. (b) Block.^{1a} (c) Kindred.^{1b}

irradiation of the lesions of mycosis fungoides. The disease is notoriously pleomorphic in its histologic manifestations, and the individual lesions tend to change from time to time. Consequently, unless a pre-treatment biopsy is made immediately prior to the start of therapy to serve as a basis for comparison of the post-treatment biopsies, it is impossible to evaluate the effects of treatment.

In the literature there are two articles on the histopathologic effect of roentgen rays on mycosis fungoides, one by Herxheimer and Hübner⁷ and the other by Pautrier.⁸ They have described the disappearance of the infiltrate after local irradiation and have expressed the belief that there was a specific effect on the mycosis cell although they did not substantiate this concept except by theorizing that the mycosis cell is embryonic in nature and consequently radiosensitive. Lubarsch and Wätjen, in their review⁹ of histopathologic observations of radiation therapy, question the validity of this explanation. The most recent and by far the most exhaustive study of the histopathologic effects of radiation, edited by Bloom,¹⁰ has refuted this time-honored but otherwise poorly substantiated theory.

From a review of the reports on the histopathologic effects of nitrogen mustard and roentgen rays on the lesions of the skin of patients with mycosis fungoides, as well as from our own study, only two facts are demonstrable, namely, that the cellularity of the tumor-like lesion decreases and that at no time is a lesion healed microscopically. To this one may add that a destructive effect on the lymphocytes, the granulocytes and, to a much less extent, the plasmacytes and a decrease in the number of mitoses in mycosis cells are demonstrable. As in a previous study on tumors of the hemopoietic organs treated with nitrogen mustard,^{6a} the amount of cellular destruction objectively demonstrated by a comparison of pretreatment and post-treatment biopsy specimens in cases of mycosis fungoides does not seem adequate to account for the clinical improvement noted. There is no more evidence to support the presence of a specific effect on embryonic cells or on reticuloendothelial cells after treatment with nitrogen mustard than after irradiation.

No severe hemorrhagic manifestations have been reported following a single course of nitrogen mustard therapy in mycosis fungoides

7. Herxheimer, K., and Hübner, H.: *Arch. f. Dermat. u. Syph.* **84**:241, 1907.

8. Pautrier, L. M.: *Strahlentherapie* **6**:257, 1915.

9. Lubarsch, O., and Wätjen, J.: *Allgemeine und spezielle pathologische Histologie der Strahlenwirkung*, in Lazarus, P.: *Handbuch der gesamten Strahlenheilkunde*, Munich, J. F. Bergmann, 1928, vol. 1, p. 304.

10. Bloom, W.: *The Histopathology of Irradiation from External and Internal Sources*, National Nuclear Energy Series (Manhattan Project Technical Section), New York, McGraw-Hill Book Co., Inc., 1948, vol. 22 I.

with the dosage of 0.1 mg. per kilogram on four successive days.³ The gelatinous aplasia of the marrow at autopsy in the case presented was far greater than had been noted following injection of nitrogen mustard at the standard dose level ^{6a} and corresponded in degree to that seen at higher dosage levels in human beings and animals and in terminal stages of Hodgkin's disease.⁶ Consequently, one cannot help but wonder whether the combination of para-aminobenzoic acid and nitrogen mustard does not have a more depressing action on the marrow than does nitrogen mustard alone. The increase in mast cells noted especially in the autopsy specimen may also have had some relation to the hemorrhagic tendency, because the mast cells have been related to the increase of a heparin-like substance in the blood after irradiation and after treatment with nitrogen mustard.¹¹

SUMMARY AND CONCLUSIONS

Within a few hours after injection of nitrogen mustard there is evidence of a degeneration of granulocytes, small lymphocytes and, to a much less extent, of plasma cells and mycosis cells. The number of mitoses in mycosis cells is greatly decreased, and in some stages mitosis is inhibited.

Objective histopathologic evidence fails to support the concept of a destructive effect of nitrogen mustard on reticuloendothelial cells or cells of embryonic nature.

Histopathologic study of the lesions after treatment has failed to account for the marked change in the lesions seen clinically.

Histologic observation failed to demonstrate a complete cure of any lesion of mycosis fungoides at any time after treatment with nitrogen mustard.

At all intervals at which a specimen was obtained for biopsy it was always possible to identify the lesion as mycosis fungoides. Treatment had produced a comparatively acellular lesion without in any way modifying the fundamental nature of the disease.

11. Jorpes, J. E.: Heparin in the Treatment of Thrombosis, New York, Oxford University Press, 1946.

BRONCHIAL ADENOMA PRODUCING AN "ALVEOLAR CELL CARCINOMA" PATTERN

J. H. CHEEK, M.D.

AND

E. E. MUIRHEAD, M.D.

DALLAS, TEXAS

IN RECENT years "alveolar cell carcinoma,"¹ "alveolar cell tumor,"² "pulmonary adenomatosis"³ and "lobar carcinoma"⁴ have been observed in man and described. A comparable process has been observed in the disease of sheep known as jaagsiekte.⁵ In these conditions the alveolar septums become partially or completely lined by cuboidal to columnar epithelium, usually of a single layer, but at times stratified in focal areas. Papillary projections into the alveolar spaces can frequently be demonstrated.

The origin of such processes has been much discussed and remains even now an unsettled matter. A recognized change in chronic pneumonitis is a cuboidal lining of the alveolar spaces. The histologic resemblance of the infectious disease of sheep, jaagsiekte, to "pulmonary adenomatosis" of man is striking, though all efforts to prove that the latter is of an infectious origin have been unsuccessful. Some have believed that perhaps these two diseases are identical and are not unlike the so-called "alveolar cell carcinoma" or "alveolar cell tumor."³ Herbut⁶ expressed the opinion that the basal layer of cells in bronchi or bronchioles breaks through the alveolar walls and extends along the lining of the alveolar spaces as cuboidal and low columnar epithelium. He could find no evidence that these cells had their origin from cells lining the alveolar spaces. Neubuerger and associates⁷ expressed the belief that the cells giving the "alveolar cell" picture have their origin from the cells which line the alveolar spaces. Because

From the Department of Surgical Pathology, Baylor University Hospital.

1. Ikeda, K.: *Am. J. Clin. Path.* **15**:50, 1945.

2. (a) Neubuerger, K. T.: *J. Thoracic Surg.* **10**:557, 1941. (b) Herbut, P. A.: *Am. J. Path.* **20**:911, 1944.

3. Simon, M. A.: *Am. J. Path.* **23**:413, 1947.

4. Silverman, G., and Angrist, A.: *Arch. Int. Med.* **8**:369, 1948.

5. Dungal, N.: *Proc. Roy. Soc. Med.* **31**:497, 1938.

6. Herbut, P. A.: *Arch. Path.* **41**:175, 1946. Herbut.^{2b}

7. Neubuerger, K. T., and Geever, E. F.; *Arch. Path.* **33**:551, 1942. Geever, E. F.; Carter, H. R.; Neubuerger, K. T., and Schmidt, E. A.: *Radiology* **44**:106, 1945. Neubuerger.^{2a}

these cells were considered independent of the bronchial lining, and because a controversy concerning them existed, these workers preferred the term "alveolar cell tumor" rather than "carcinoma."

There has also been considerable discussion concerning the histogenesis of the "bronchial adenoma," apparently because of its variable histologic picture. Graham and Womack⁸ found that different areas revealed different patterns within the same tumor. The close resemblance of some adenomas to fetal lung suggested that these tumors could have arisen from endobronchial buds which had failed to develop. The origin of bronchial adenoma from peribronchial mucous glands or from the basal cell layer of the bronchial lining has been thought to be the basis for the pattern an adenoma may show.⁹ Thus, according to this view, adenoma arising from the basal layer of cells may give rise to tumors composed of small cells, with scanty cytoplasm and small, round, dark-staining nuclei, which tend to arrange themselves in clusters or cords, and adenoma from glands may show a tumor with varying glandular arrangement and composed of large polygonal or columnar cells with wide zones of pale, pink-staining, finely granular cytoplasm and with fairly large vesicular to dark-staining nuclei. Attention has been repeatedly directed to the close resemblance of adenoma to mixed tumors of the salivary glands, carcinoid of the appendix, basal cell carcinoma of the skin, so-called cylindroma and even to islet cell tumors of the pancreas and parathyroid adenoma. In fact, bronchial adenoma has been at times classified according to these similarities. Graham and Womack⁸ accounted for the mixed tumor appearance on the basis that the stroma may become dominated by cells of mesoblastic origin giving rise to connective tissue, muscle, cartilage, fat and bone. Holly¹⁰ used the term "carcinoid" because the cells of the tumor, the growth characteristics and the cellular arrangement seem to be identical with those of carcinoid of the appendix. Stout¹¹ expressed the belief that oncocytes, special cells found in the wall of bronchial ducts and glands, may be the origin, since he has been able to demonstrate them in some of the adenomas.

We wish to report 2 representative cases of bronchial adenoma in which the histologic picture at the advancing edge of the tumor or elsewhere in the lung has been identical with that seen in pulmonary adenomatosis or the so-called alveolar cell carcinoma or tumor. One of these cases (no. 2) was revealed in a surgical specimen, and the other, at autopsy.

8. (a) Graham, E. A., and Womack, N. A.: *J. Thoracic Surg.* **14**:106, 1945.
(b) Womack, N. A., and Graham, E. A.: *Arch. Path.* **26**:165, 1938.

9. (a) Fried, B. M.: *Arch. Int. Med.* **79**:291, 1947. (b) Sano, M. E., and Meade, R.: *Arch. Path.* **43**:235, 1947.

10. Holly, S. W.: *Mil. Surgeon* **99**:528, 1946.

11. Stout, A. P.: *Arch. Path.* **35**:803, 1943.

REPORT OF CASES

CASE 1.—A 44 year old white woman entered the Baylor University Hospital, Oct. 29, 1947, with shortness of breath and coughing of blood for a period of three months. Previously she had been in good health. She had lost 8 to 10 pounds



Fig. 1 (case 1).—A typical bronchial adenoma with the so-called carcinoid pattern in the wall of the larger bronchus. Two mucus-filled spaces can be seen in the left upper corner.

Fig. 2 (case 1).—The edge of the tumor in figure 1 showing a mixture of carcinoid and mucus-secreting glandular elements with a greater amount of mucus-secreting glandular structures present. Beginning of lining of alveolar walls can be detected.

(3.5 to 4.5 Kg.) in weight and had fever (temperature, 103 F.) with no chills. Deep inspiration caused cough, and this exaggerated the dyspnea. There was no family history of tuberculosis or of tuberculous contacts. The patient was well nourished and well developed. The blood pressure was 110 systolic and 80 diastolic. The pulse rate was 145; the respiratory rate, 22. She was more comfortable lying flat in bed, but even so her respiration was labored. No cyanosis was noted. There was a deep, nonproductive cough. The chest expanded equally in all directions. Flatness was noted over the upper part of the chest, both anteriorly and posteriorly, and the breath sounds were high pitched over these areas. There was increased dyspnea with coughing when the patient tried to sit up in bed.

A roentgenogram of the chest revealed extensive miliary infiltrations, and it was thought that there was an enlargement of the hilar lymph nodes.

Course in Hospital.—Skin tests with coccidioidin and histoplasmin gave negative results. The temperature varied from 98.6 to 100 F.; the pulse rate, from 110 to 158, and respirations occurred at a rate of between 18 and 54 per minute. Numerous examinations of sputum revealed no acid-fast bacilli. Oxygen therapy was started and intramuscular injections of streptomycin were given, but the patient's condition showed little change. She gradually grew worse and died on the fifth hospital day. Pneumonitis, possibly tuberculous, and carcinomatosis were the clinical impressions.

Autopsy.—The lungs were shrunken and firm in consistency. The surfaces were finely nodular, with the nodules varying in size from 1 to 5 mm. and of a grayish yellow color. Throughout both lungs were scattered nodules of a similar size and color. Small nodules of a similar type were noted on the surface of the liver and within the hepatic parenchyma. Nodules were also found within the adrenal glands, the parietal pericardium and mediastinal lymph nodes. At the bifurcation of the bronchus to the lower lobe of the right lung was a grayish red polypoid mass, measuring 1 by 0.5 cm., which was friable and which almost completely obstructed the bronchus. It extended for a few millimeters into both limbs of the bifurcation.

The tumor within the bronchus to the lower lobe of the right lung was composed of small, dark-staining cells of uniform size. The histologic picture was that of the carcinoid tumor (fig. 1 and 2). Other areas of the tumor were composed of tall, columnar, mucus-secreting epithelium lining spaces containing mucus (fig. 3). At one margin of the tumor was a transition from the carcinoid picture to the alveolar cell arrangement. Here again the cells were tall, columnar, with oval to round vesicular nuclei and pale, pink-staining cytoplasm which in many instances showed secretory vacuoles. These cells were growing into and lining the alveolar spaces. Sections from the areas of metastases (fig. 4) showed similar tumor cells lining alveolar spaces; these spaces often contained a mucinous material.

CASE 2.—M. M., a 53 year old woman, was admitted to the hospital with the history that a routine roentgenogram of the chest in March 1947 revealed a "spot" in the upper lobe of the right lung. The patient continued to perform her normal duties and continued to have roentgen examinations of the chest every three months. In July 1948 there was noted a slight increase in the size of the lesion, and on the basis of this finding an operation was recommended. During this period of time she had been asymptomatic. Her past history was noncontributory except for serious pneumonia at the age of 6 and hysterectomy in 1943. She was a well nourished woman with a blood pressure reading of 120 systolic and 78 diastolic; small, firm, nontender nodes were palpated in the left cervical region. Roentgenograms revealed a circumscribed lesion near the periphery of the base of the upper lobe of the right lung. On Aug. 2, 1948 (sixteen months after the discovery of the lesion) thoracotomy was done. A tumor was found within the anterior segment of the upper lobe of the right lung, measuring roughly 2 cm. in diameter. Puckering

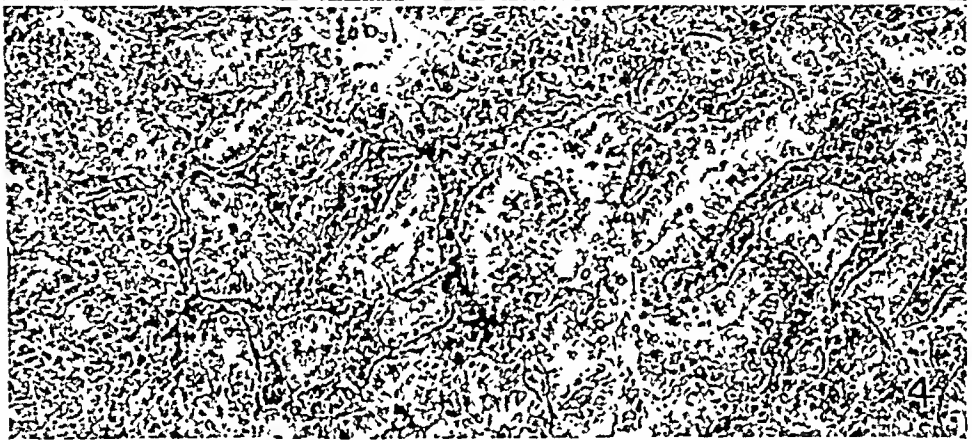
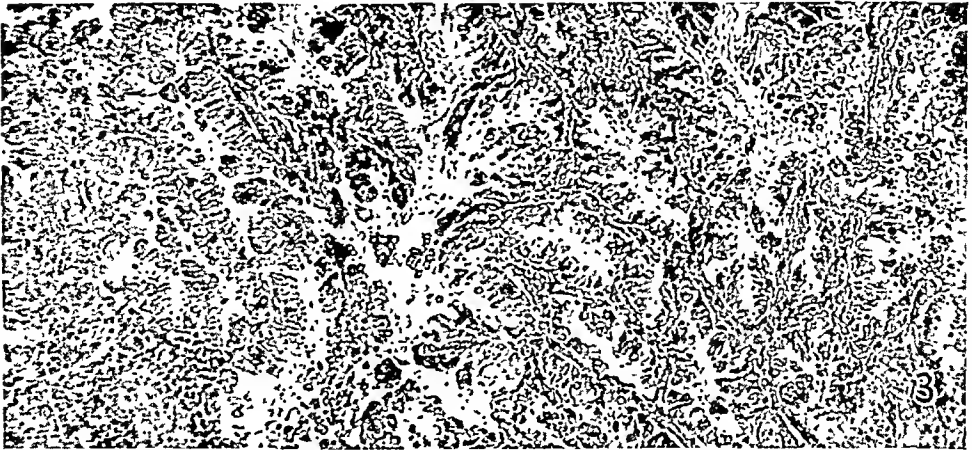


Fig. 3. (case 2).—The glandular, mucus-secreting make-up of the so-called "alveolar cell" carcinoma at its center is demonstrated.

Fig. 4 (case 1).—Papilliferous structure of a metastatic nodule of the opposite lung. Lining of alveolar walls with columnar cells of a mucus-secreting type is evident. This pattern was observed not only as a transition from the adenoma but elsewhere in both lungs.

Fig. 5 (case 2).—The advancing edge of the tumor. Utilization of the lung structure as a scaffold for growth and lining of the alveolar walls are demonstrated.

of the overlying pleural surface was noted. There were no enlarged hilar nodes. The tumor was excised widely, and the postoperative course was satisfactory.

Grossly, the lesion on section was fairly well circumscribed and measured 2 cm. in diameter. It was composed of a moderately dense, mottled, grayish yellow tissue in which were multiple minute dark areas considered to be pigment.

Microscopically (fig. 4), the tumor was composed of cuboidal to tall columnar or polyhedral cells, which formed glandular structures at the center. These cells had fairly large oval to round vesicular nuclei and pale, pink-staining, finely granular cytoplasm. In some areas a piling up or pseudostratification was noted, as were papillary projections of cells extending into some of the alveolar spaces. At the periphery (fig. 5) these cells were growing out into and lining the alveolar spaces. In some areas one side of the alveolar septum was lined by these cells while the opposite side showed no cells.

COMMENT

It seems that the so-called alveolar cell pattern is not pathognomonic of any one process, since it can be seen in infectious processes, in relatively benign tumors and in cancers, as well as in the metastatic lesions of the lungs. It has been previously pointed out that metastatic adenocarcinoma can produce an alveolar cell pattern in the lung.⁶ We have seen this pattern in adenocarcinoma of the breast, the stomach, the prostate, the kidney and the pancreas and in one transitional cell carcinoma of the bladder. It is of further interest that in certain cases carcinoma of the lung has in one area a definite squamous cell and in another area a clearcut alveolar cell carcinoma pattern. In our cases the alveolar cell pattern was well demonstrated, with cuboidal and columnar cells growing out into and lining the alveolar spaces in a uniform manner and, in focal areas, producing papillary projections.

Bronchial adenoma occurs more frequently in women and is usually described as observed in the third and fourth decades of life, whereas bronchiogenic carcinoma occurs in the fifth and sixth decades and is more prevalent in men. The question of cancer with regard to bronchial adenoma continues to be a serious one. Some feel that all of these tumors should be considered cancerous but at the same time should be separated from bronchiogenic carcinoma.¹² Cases in which metastases occurred are reported in support of this view. Others consider the tumor relatively benign and only occasionally cancerous.¹³ In our experience bronchial adenoma is locally invasive and may produce metastases in nodes, the opposite lung and elsewhere.

The morbidity, the symptoms and the signs associated with bronchial adenoma usually are due to bronchial obstruction and to secondary

12. Harrington, S. W.; Moersh, H. J.; Tinney, W. S.; McDonald, J. R., and Clagett, O. T.: *Proc. Staff Meet., Mayo Clin.* **21**:409, 1946. Womack.^{8b} Anderson, W. M.: *J. Thoracic Surg.* **12**:351, 1943. Adams, W. E.; Steiner, P. E., and Bloch, R. G.: *Surgery* **11**:503, 1942.

13. Allen, I. V.: *Canad. M. A. J.* **55**:498, 1946. Fried.^{9a} Sano and Meade.^{9b} Holly.¹⁰

inflammatory processes, which may suggest some other pulmonary disease. We believe that in case 2 the tumor was obviously a relatively benign bronchial adenoma, because of its long course and lack of cancerous change. Lack of symptoms referable to it can probably be accounted for on the basis of its peripheral location with subsequent lack of bronchial obstruction and secondary inflammatory changes. The tumor represented a bronchial adenoma the origin of which could not be identified but the cells of which suggest that it originated from peribronchial glands. It was composed of cuboidal to columnar cells, which were growing out into and lining the alveolar spaces, giving a definite "alveolar cell carcinoma" pattern. In case 1 a bronchial adenoma with the typical carcinoid picture was demonstrated. At its periphery was a transition into a tumor composed of taller cells lining alveolar spaces and producing the "alveolar cell carcinoma" pattern. The tumor had produced widespread pulmonary extensions and metastases to the hilar nodes, the pericardium, the liver and the adrenal glands, producing in these sites a pattern like that of the original tumor.

SUMMARY

The histologic pictures of pulmonary adenomatosis and "alveolar cell carcinoma" have been discussed, after a brief review of the ideas of the origins of these tumors. The usual arrangement or picture of bronchial adenoma and its origin have been discussed. Two cases of bronchial adenoma have been reported in which the tumor produced an "alveolar cell carcinoma" or adenomatosis pattern. Both tumors were of low grade histologic appearance. One was locally invasive only; the other was locally invasive and produced widespread pulmonary extensions and distant metastases.

EXPERIMENTAL ATHEROSCLEROSIS

X. The Effect of Desoxycorticosterone Acetate on the Cholesterol Content of the Blood, the Aorta and the Liver of the Rabbit

MAURICE BRUGER, M.D.

AND

BERTRAND E. LOWENSTEIN, M.D.

NEW YORK

HYPERCHOLESTEREMIA and atherosclerosis develop readily in the cholesterol-fed rabbit,¹ but their severity can be affected by a number of factors. Since hypercholesteremia and atherosclerosis represent abnormalities of metabolism, it is logical to expect that the endocrine system (one of the major governors of metabolism) should exert considerable influence on these effects, and this expectation has, in fact, been borne out experimentally. Thus, the administration of either an inorganic iodide or thyroid gland substance inhibits the deposition of cholesterol in the arterial tree,² although injection of anterior pituitary thyrotropic extract not only fails to inhibit but actually enhances the tendency toward atherosclerosis.³ The steroid hormones also are effective. Testosterone propionate and estradiol dipropionate inhibit hypercholesteremia and atherosclerosis in the female rabbit but have no protective action either in the male⁴ or the castrate female.⁵ In birds blood lipids are known to rise after the

From the Medical Research Laboratory, Department of Medicine, Post-Graduate Medical School, New York University-Bellevue Medical Center.

1. Anitschkow, N.: *Beitr. z. path. Anat. u. z. allg. Path.* **56**:379, 1913. Wacker, L., and Hueck, W.: *München. med. Wchnschr.* **60**:2097, 1913; *Arch. f. exper. Path. u. Pharmakol.* **74**:416, 1913. Duff, G. L.: *Arch. Path.* **20**:81, 1935.

2. Leary, T.: *Arch. Path.* **21**:459, 1936; *J. A. M. A.* **105**:475, 1935. Bruger, M., and Rosenkrantz, J. A.: *J. Clin. Endocrinol.* **2**:176, 1942. Murata, M., and Kataoka, S.: *Verhandl. d. japan. path. Gesellsch.* **7**:27, 1917. Liebig, H.: *Arch. f. exper. Path. u. Pharmakol.* **159**:265, 1930; **175**:409, 1934. Seel, H., and Creuzberg, G.: *ibid.* **161**:674, 1931. Turner, K. B.: *J. Exper. Med.* **58**:115, 1933. Friedland, I. B.: *Ztschr. f. d. ges. exper. Med.* **87**:683, 1933. Page, I. H., and Bernhard, W. G.: *Arch. Path.* **19**:530, 1935. Turner, K. B., and Khayat, G. B.: *J. Exper. Med.* **58**:127, 1933.

3. Bruger, M., and Fitz, F.: *Arch. Path.* **25**:637, 1938.

4. Ludden, J. B.; Bruger, M., and Wright, I. S.: *Arch. Path.* **33**:58, 1942.

5. Bruger, M.; Wright, I. S., and Wiland, J.: *Arch. Path.* **36**:612, 1943.

administration of estrogens,⁶ although testosterone, progesterone, androstenedione and desoxycorticosterone are all without effect.⁷

The interrelationship between the adrenal cortex and cholesterol metabolism has also been studied. Thus, Maranon and Collazo⁸ noted that in the presence of a hypofunctioning adrenal cortex, hypocholesteremia developed, while increased adrenal activity produced hypercholesteremia. These observers also reported that the administration of adrenal cortex extract to either man or dog increased the blood cholesterol. Fiandacca and Capizzi⁹ used three fractions of whole adrenal cortex and found that one fraction corrected hypocholesteremia, another lowered the blood cholesterol, while the third had no influence on the blood level of this lipid. Villela¹⁰ claimed that the administration of desoxycorticosterone lowered the plasma cholesterol in normal guinea pigs.

More recently, Long and his collaborators¹¹ have shown that if the adrenal cortex is stimulated with anterior pituitary adrenotropic extract, epinephrine or nociceptive stimuli, the stimulation is followed by an impressive decrease in the cholesterol content of the adrenal gland. Long postulated that this change may represent conversion of cholesterol into the characteristic adrenal cortical steroids.

On the other hand, Baumann and Holly¹² studied the effect of unilateral and bilateral adrenalectomy on the blood cholesterol and lipid phosphorus of rabbits and observed no significant changes. Randles and Knudson¹³ found that the removal of the adrenal glands of rats did not alter the blood cholesterol level, while Entenman and associates,⁷ working with chickens, failed to raise the blood cholesterol, total fatty acid or phospholipid values by injecting desoxycorticosterone acetate. Harrop and his co-workers¹⁴ were unable to influence the blood cholesterol of either man or dog by injecting adrenal

6. Lorenz, F. W.; Chaikoff, I. L., and Entenman, C.: *J. Biol. Chem.* **126**:763, 1938. Zondek, B., and Marx, L.: *Arch. internat. de pharmacodyn. et de therap.* **61**:77, 1939. Landauer, W.; Pfeiffer, C. A.; Gardner, W. U., and Man, E. B.: *Proc. Soc. Exper. Biol. & Med.* **41**:80, 1939.

7. Entenman, C.; Lorenz, F. W., and Chaikoff, I. L.: *J. Biol. Chem.* **134**:495, 1940. Kochakian, C. D.; MacLachlan, P. L., and McEwen, H. D.: *ibid.* **122**:433, 1938.

8. Maranon, G., and Collazo, S. A.: *Rev. franç. d'endocrinol.* **13**:1, 1935.

9. Fiandacca, S., and Capizzi, I.: *Endokrinologie* **14**:316, 1934.

10. Villela, O.: *Hospital* **19**:41, 1941.

11. Long, C. N. H.: *Federation Proc.* **6**:461, 1947.

12. Baumann, E. J., and Holly, B. M.: *J. Biol. Chem.* **55**:457, 1923.

13. Randles, F. S., and Knudson, A.: *J. Biol. Chem.* **76**:89, 1928.

14. Harrop, G. A., Jr.; Swingle, W. W., and Pfiffner, J. J.: *J. Biol. Chem.* **92**:lvi, 1931.

cortex extract. Furthermore, Raab and associates¹⁵ were unable to influence the course of experimental atherosclerosis in the rabbit by either adrenalectomy or by the administration of either adrenal cortex extract or anterior pituitary adrenotropic extract.

While the bile acids are not hormones, they possess a steroid structure, and it therefore seems worth while to mention their effects

TABLE 1.—*Cholesterol Contents of Blood, Aorta and Liver of 24 Young Female Rabbits Fed 1.0 Gm. of Cholesterol Three Times per Week for Twelve Weeks*

Rabbit	Whole Blood Cholesterol (Mg. per 100 Cc.)														Liver Cholesterol, Mg. per 100 Gm. Wet Wt.	Aorta Cholesterol, Mg. per 100 Gm. Dry Wt.
	Total Cholesterol							Ester (per Cent of Total)								
	0 Wk.	2 Wk.	4 Wk.	6 Wk.	8 Wk.	10 Wk.	12 Wk.	0 Wk.	2 Wk.	4 Wk.	6 Wk.	8 Wk.	10 Wk.	12 Wk.		
14.....	80	610	690	850	940	940	670	13	53	40	55	58	48	52	2,540	3,470
15.....	170	250	250	200	820	1,340	1,070	29	44	42	54	52	56	82	1,890	810
16.....	100	210	410	190	550	890	690	19	24	28	48	48	47	59	2,400	690
17.....	120	380	290	610	870	1,010	...	17	33	41	52	54	47	..	980	4,370
18.....	99	120	160	280	710	1,250	1,010	13	30	28	62	48	51	56	3,710	2,930
19.....	96	360	190	120	390	1,250	1,170	20	23	11	23	57	70	68	2,840	1,610
20.....	100	210	210	490	640	940	940	18	32	12	44	53	67	51	2,350	1,530
21.....	130	180	130	450	750	850	630	17	31	31	55	57	58	61	1,510	3,460
22.....	100	180	340	360	660	960	870	700	2,640
23.....	130	300	250	220	250	240	600	450	650
24.....	110	380	560	940	820	890	630	21	54	46	51	50	48	40	940	9,620
25.....	110	220	390	330	420	660	660	17	39	47	23	42	50	30	1,010	1,650
26.....	140	290	500	600	680	770	830	25	37	44	40	41	42	41	640	2,840
27.....	99	210	350	420	750	1,100	1,100	34	54	44	47	41	41	51	510	3,380
28.....	130	340	180	150	280	260	270	22	55	53	47	50	36	28	440	930
29.....	99	370	630	670	630	990	1,140	25	41	62	38	50	47	36	720	3,540
30.....	85	260	270	420	710	550	...	22	49	66	47	51	29	..	1,120	1,160
70.....	64	190	520	730	890	1,100	850	9	39	64	63	70	55	44	1,980	5,230
71.....	64	200	68	160	250	200	280	14	32	31	59	44	30	24	960	510
72.....	92	300	640	850	780	940	960	11	55	57	63	56	52	41	1,700	6,000
73.....	69	180	480	510	580	730	750	14	21	71	69	55	57	50	1,280	6,010
74.....	66	200	210	420	310	400	350	13	38	53	71	45	43	50	1,010	1,550
75.....	64	630	800	940	1,010	990	800	9	57	64	71	19	60	35	2,080	3,320
76.....	63	680	830	820	960	1,140	1,070	17	55	57	57	19	45	43	2,470	5,210
Average.....	99	300	390	490	650	850	790	18	41	45	52	48	49	47	1,510	3,050

here. Simultaneous feeding of bile salts and cholesterol to mice greatly augments the amount of cholesterol deposited in the liver over that found if only cholesterol is fed.¹⁶ In work published from this laboratory, it was shown that the cholesterol content of the blood and the aorta of the cholesterol-fed rabbit could be greatly increased if cholic

15. Raab, W.; Wachstein, M., and Straubb, S.: *Ztschr. f. d. ges. exper. Med.* **102**:212, 1937.

16. Hummel, R.: *Ztschr. f. physiol. Chem.* **185**:105, 1929. Loeffler, K.: *ibid.* **178**:186, 1928.

acid or glycocholic acid was added to the dietary regimen.¹⁷ This effect was ascribed to the role of bile acids in favoring absorption of cholesterol from the gastrointestinal tract and not to the related chemical configuration.

In view of the fact that these studies do not permit definite conclusions regarding the influence of adrenal cortical steroids on experi-

TABLE 2—*Effect of 1.5 to 2.5 Mg. of Desoxycorticosterone Acetate Injected Triweekly on the Cholesterol Content of Blood, Aorta and Liver of 26 Young Female Rabbits Fed 10 Gm. of Cholesterol Three Times per Week for Twelve Weeks*

Rabbit	Whole Blood Cholesterol (Mg. per 100 Cc.)														Liver Cholesterol, Mg. per 100 Gm. Wet Wt.	Aorta Cholesterol, Mg. per 100 Gm. Dry Wt.
	Total Cholesterol							Ester (per Cent of Total)								
	0	2	4	6	8	10	12	0	2	4	6	8	10	12		
	Wk.	Wk.	Wk.	Wk.	Wk.	Wk.	Wk.	Wk.	Wk.	Wk.	Wk.	Wk.	Wk.	Wk.		
1. .	81	390	410	580	590	590	430	26	45	62	47	41	51	48	1,550	7,210
3.....	100	680	770	1,040	1,010	780	770	22	47	52	55	61	51	56	2,160	5,860
4.....	92	530	550	540	670	24	45	56	..	54	910	760
8.....	96	320	590	730	1,500	1,440	...	19	49	48	66	67	61	..	3,530	1,790
9.....	110	290	490	600	830	710	390	21	37	43	55	61	59	..	890	2,020
10.....	91	170	380	250	440	670	..	11	34	27	53	54	44	..	780	610
11.....	110	200	240	320	400	500	180	18	23	33	41	54	47	30	510	1,240
12.....	92	300	480	630	780	890	670	20	36	42	66	57	60	67	1,650	3,940
13....	110	200	130	130	420	360	630	32	..	21	27	..	56	70	1,350	1,940
31....	96	160	190	140	160	260	550	670
32.....	120	190	180	270	210	190	350	740	1,270
33....	100	270	320	480	470	490	600	1,540	1,860
34....	85	210	200	270	220	260	280	490	990
35....	110	190	380	320	290	450	620	570	3,640
36.	120	280	260	490	460	430	660	650	1,820
37.....	100	170	230	150	260	600	710	610	1,510
38. .	100	280	240	340	350	520	440	880	1,750
77....	89	310	390	770	990	1,040	1,040	23	32	54	63	64	60	42	3,130	2,710
78....	78	96	210	360	570	500	560	14	54	60	54	77	53	28	1,030	930
79	71	420	190	650	770	850	600	20	55	..	51	62	51	60	1,720	1,090
80. . .	59	130	200	230	290	430	340	10	35	66	57	56	49	45	1,470	510
81. . .	79	830	490	630	940	1,070	960	11	51	71	81	64	55	40	2,500	9,090
82.. . . .	67	310	74	320	690	690	530	16	43	48	49	46	52	34	1,830	630
83.....	130	300	490	460	630	1,070	1,180	14	56	45	63	60	59	42	1,900	4,050
84	74	360	400	660	750	750	750	22	49	41	54	56	60	39	2,000	6,630
85.	73	100	150	240	380	640	540	21	20	43	47	..	52	38	1,440	1,000
Average... ..	93	300	330	440	580	650	610	19	42	48	55	58	54	46	1,400	2,520

mental atherosclerosis, the present experiment was undertaken to ascertain the effect of desoxycorticosterone acetate on the blood cholesterol levels and on the cholesterol content of the aorta and the liver of the young female rabbit fed cholesterol.

17. Member, S.; Bruger, M., and Oppenheim, E.: Arch. Path. 38:210, 1944.

METHODS AND MATERIAL

Fifty female rabbits (Wistar strain) aged 3 to 5 months were employed. Twenty-four animals served as controls: 1 Gm. of cholesterol was added to the basal diet¹⁸ three times a week for twelve weeks. In the experimental group, 1.5 to 2.5 mg. of desoxycorticosterone acetate¹⁹ was administered intramuscularly to 26 rabbits three times a week on days when cholesterol was fed. The total cholesterol and the cholesterol esters of the whole blood were determined²⁰ every two weeks by Bloor's method as modified by Sackett.²¹ At the end of the experimental period, the animals were killed, and the aorta and the liver were analyzed for cholesterol by the procedures previously described from this laboratory.²²

RESULTS

The detailed findings appear in tables 1 and 2. It is apparent that parenteral administration of desoxycorticosterone acetate had little or no effect on the rise of blood cholesterol levels during the first eight weeks. Toward the end of the experimental period there was a slight inhibition of the elevation of blood cholesterol. The ester percentage of total cholesterol was not significantly different in the two groups. Table 3 represents a statistical analysis of the effect of desoxy-

TABLE 3.—*Statistical Analysis of Results*

Group	Specimens	Liver Cholesterol			Aorta Cholesterol		
		Mean	α *	t †	Mean	α *	t †
Cholesterol.....	24	1,510	± 860	3,050	$\pm 2,150$
Cholesterol + desoxycorticosterone acetate.....	26	1,400	± 790	0.46	2,520	$\pm 2,270$	0.80

* Standard deviation $\alpha = \sqrt{\frac{\sum (d^2)}{N}}$ where $\sum (d^2)$ represents the summation of the squares of the individual deviations from the mean and N the number of determinations.

$$\dagger t = \frac{\overline{X_1} - \overline{X_2}}{\sqrt{\frac{N_1 \alpha_1^2 + N_2 \alpha_2^2}{N_1 + N_2 - 2}}} \cdot \sqrt{\frac{N_1 N_2}{N_1 + N_2}} \text{ for a small series and } t \text{ was checked against Fisher's}$$

tables. In this formula $\overline{X_1}$ represents the mean for the cholesterol-fed animals, $\overline{X_2}$ the mean for the cholesterol + desoxycorticosterone acetate group, N_1 the number of specimens in the cholesterol group, N_2 the number of specimens in the cholesterol + desoxycorticosterone acetate series, α_1 the standard deviation of the cholesterol-fed animals and α_2 the standard deviation of the cholesterol + desoxycorticosterone acetate group. In both the liver and the aorta cholesterol series the t values do not indicate a significant difference.

corticosterone acetate on the deposition of cholesterol in the liver and the aorta. In the control group the average amount of cholesterol in the liver was 1,510 mg. per hundred grams and the average amount in the aorta was 3,050 mg., while in

18. The basal diet was Ralston Purina® rabbit chow, which contains a mixture of grains and alfalfa hay supplemented with vitamins and minerals.

19. The desoxycorticosterone used was that marketed as per corten (Ciba)® 0.3 to 0.5 cc.

20. These determinations were made with the technical assistance of Samuel Member, B.S.

21. Sackett, G. E.: J. Biol. Chem. 64:203, 1925.

22. Rosenkrantz, J. A., and Bruger, M.: Arch. Path. 42:81, 1946. Bruger and Fitz.³

the treated group the averages were 1,400 mg. and 2,520 mg. per hundred grams, respectively. However, neither of these small differences for the small number of animals were statistically significant.

COMMENT

This study has demonstrated that desoxycorticosterone acetate has little if any inhibitory influence on the development of hypercholesteremia or on the deposition of cholesterol in the liver or the aorta of the normal young female rabbit fed cholesterol. In view of the conflicting results reported by previous investigators and in view of the fact that desoxycorticosterone acetate is only 1 of 28 compounds thus far isolated from the adrenal cortex²³ it is not permissible to interpret the negative results of this experiment as indicating that the adrenal cortex has no part to play in cholesterol metabolism. Particularly is this true since desoxycorticosterone acetate is primarily involved in mineral and water metabolism,²⁴ while the other steroids of the adrenal cortex affect the metabolism of organic substances.

CONCLUSIONS

Parenteral administration of desoxycorticosterone acetate has little or no effect on whole blood cholesterol levels in rabbits fed cholesterol.

Desoxycorticosterone acetate does not prevent the development of atherosclerosis of the aorta or the deposition of cholesterol in the liver of the rabbit under the conditions of the experiment.

23. Kendall, E. C.: Function of the Adrenal Cortex, in *Glandular Physiology and Therapy*, Chicago, American Medical Association, 1942, chap. 18, p. 273. Reichstein, T., and Shoppee, C. W., in Harris, R. S., and Thimann, K. V.: *Vitamins and Hormones*, New York, Academic Press, Inc., 1943, vol. 1, p. 345. Kenyon, A. T.: *Surgery* **16**:194, 1944.

24. Thorn, G. W.; Engel, L. L., and Eisenberg, H.: *J. Exper. Med.* **68**:161, 1938. Ferrebee, J. W.; Ragan, C.; Atchley, D. W., and Loeb, R. F.: *J. A. M. A.* **113**:1725, 1939.

SILVERING OF LEPRA BACILLI IN TISSUES

F. LEÓN BLANCO, M.D.

HABANA, CUBA

AND

G. L. FITE, M.D.

CARVILLE, LA.

ALTHOUGH silver impregnations of tissues have been used previously¹ for the study of nerve changes in leprosy, and for the demonstration of reticulum, apparently silvering of the bacilli has not been observed. With the methods used by us, lepra bacilli have been found to be demonstrated routinely and effectively in biopsy material in more than 100 cases of leprosy.

PROCEDURES

The methods used have been those of Jahnelt,² Levaditi,³ and Manouélian,⁴ modified in the manner to be described. Jahnelt's method is longer and more tedious, but it has been found particularly effective, giving beautiful results in tissues fixed in formaldehyde or Bouin's fluid for as long as one year. The modification employed is, briefly, as follows:

1. Fix tissue in 4 per cent formaldehyde solution or Bouin's fluid for two weeks or more.
2. Place in pyridine for one to three days.
3. Wash in several changes of distilled water for twenty-four hours.
4. Fix in 4 per cent formaldehyde for four days.
5. Wash in several changes of distilled water for twenty-four hours.
6. Place in 95 per cent alcohol, several changes, for three to eight days.
7. Wash in distilled water until pieces of tissue sink to the bottom of the container.
8. Treat with aqueous silver nitrate (0.5 per cent in distilled water) in the dark at 37 C. for five to eight days. A copious amount of the solution should be used.
9. Wash in distilled water ten minutes to remove excess silver.

From the Patronato para la profilaxis de la lepra y sífilis, Habana, Cuba, and the United States Marine Hospital, Carville, La.

1. Takino, M.: *Acta scholae med. univ. imp. in Kioto* **13**:1, 1930.
2. Jahnelt, F.: *München. med. Wchnschr.* **67**:932, 1920.
3. Mallory, F. B.: *Pathological Technique*, Philadelphia, W. B. Saunders Company, 1938, p. 193.
4. Manouélian, Y.: *Compt. rend. Acad. d. sc.* **190**:332, 1930.

10. Reduce for two days in a solution prepared as follows: pyrogallie acid, 4 Gm.; 37 per cent formaldehyde solution, 5 cc.; distilled water, 95 cc.

11. Dehydrate, clear, embed, section and mount without further staining, by any routine method.

RESULTS

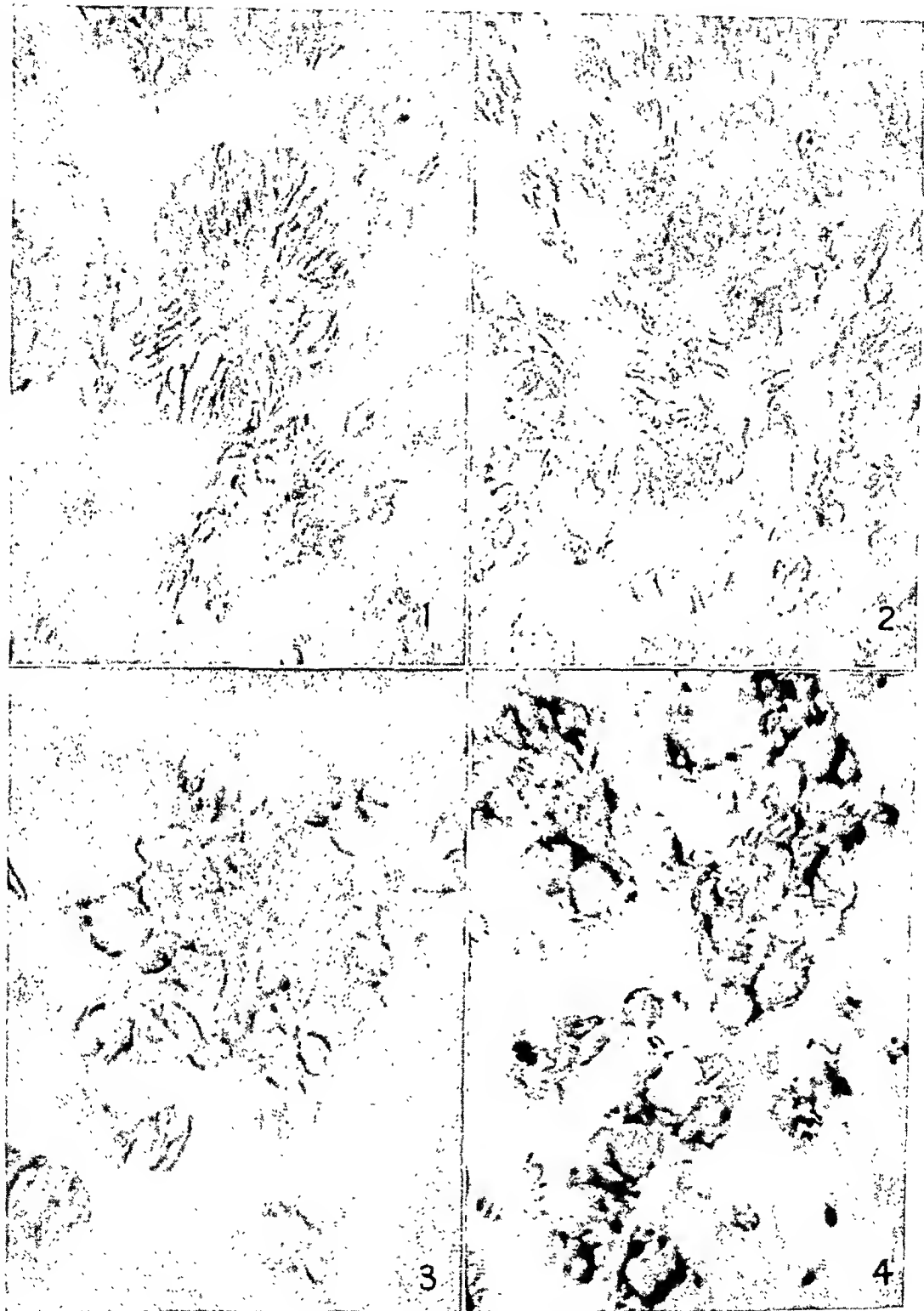
Lepa bacilli appear in various shades of black and brown, the majority in an intense black or blue-black. Differences of intensity are discussed later. Other structures, such as melanin and keratohyaline granules, are impregnated but are not confusing except close to the epidermis.

Because this method involves prolonged treatment of the whole tissue, the bacilli are extremely well fixed and are little or not at all disturbed by subsequent embedding and sectioning. The results, it is believed, produce a wholly true picture of the distribution and appearance of the bacilli in the tissues. Ordinary methods of revealing acid-fast bacilli and methods of silvering frozen sections produce much distortion, due to shrinkages, with bacilli frequently found in positions in which they could not reasonably occur. This distortion is absent from the embedded silvered preparations. Although the nuclei of the cells are not always well demonstrated, the relation of bacilli and cells is nonetheless well shown.

BACILLI IN THE MOST HIGHLY ACTIVE LESIONS

Such lesions are recognized histologically both from the large numbers of organisms present and from the character of the cells containing the organisms. These are not the vacuolated cells of the older leproma but are fairly simple macrophages of a wide variety of shapes and forms. In ordinary sections the parts of the cell occupied by the bacilli can be identified only by hollow areas in the cytoplasm which are not distinct vacuoles. Indeed, the numbers of organisms present would not be suspected, with the abundant and well stained cytoplasm.

In these lesions bacilli are seen in characteristic form (see figures 1 to 4). They occur in streams, or parallel columns, and in larger cells they radiate from the central part of the cell. They look not at all like the usually described "cigar packet" or "bundle" but resemble a small colony of a fungus with hyphae streaming out from a central point. Bacilli are not matted together but are separated by a well defined uniform narrow space. It becomes clear that the appearance of these fresh active lesions when stained with fuchsin methods is highly artefactual. In the latter case organisms are matted together and distorted in various ways. Hansen's original description (1874) of bacilli "crossing each other at very sharp angles" would not apply in this acute phase. This type of bacillary arrangement is limited to those lesions



Figs. 1 to 4.—Development of the vacuolated cells. Figures 1 and 2 illustrate the disposition of lepra bacilli in fresh active lesions. In figure 1 the bacilli radiate from the central part of the cell, which is occupied by the nucleus. Figure 3, from the same section as figure 1 shows an older area, with beginning vacuolation and bacilli curved about the vacuoles. Figure 4 shows a still older lesion with most of the bacilli ranged about vacuoles, or spread apart by the developing vacuoles, and moderate pleomorphism of the bacilli.

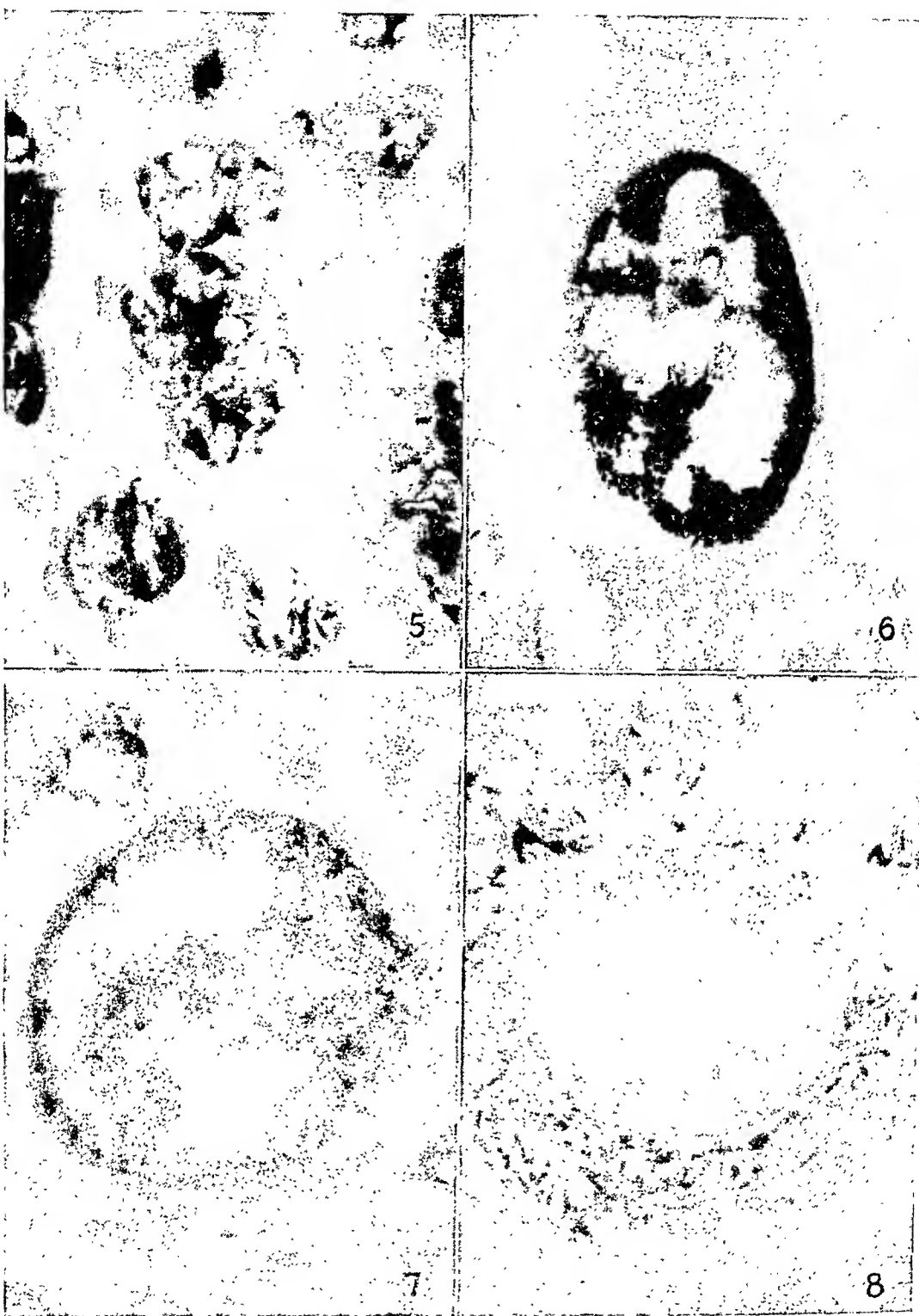
in which both bacilli and cells have been increasing in numbers, for which reason it is of especial importance. These are the growing, young bacilli.

In these lesions the bacilli appear quite long and nearly straight. They are uniformly, but not too deeply, silvered. They show little or no beading, although occasionally there is some greater condensation of the silver at the extremities of the organisms. The manner of the division of the organisms cannot be truly determined from a study of sections, but the impression is gained that in this case bacillary cell division must take place by transverse fission.

THE CHRONIC LEPROUS GRANULOMA

All stages between the highly and chronically active lesions are recognized. Of all lepromas that come under the microscope, the majority are of the chronic type, in which various degrees of vacuolation of the cells occur. There exists in leprology a popular misconception of the nature of these vacuoles, it being assumed frequently that they are the spaces occupied by the bacilli. This is not the case, as the silver impregnations show plainly. The vacuolated cells display a wide variation in size, the smallest with a single centrally placed nucleus, the larger with several nuclei anywhere in the cell. They are enmeshed in a reticulated, vascularized connective tissue framework, and in the silvered preparations the individual cell margin is demonstrated to a degree rarely observed in ordinary preparations. The bacilli are arranged in haphazard fashion in the cells. They lie between the vacuoles in what little cytoplasm is left in the cell, and the vacuoles are plainly seen as hollows between the meshwork of bacilli. The nucleus of the cell, which is commonly unsilvered by Jahnke's method but shown by Levaditi's, appears as a round space, with the bacilli lying closest to it markedly curved, their concave borders facing it. Bacilli in these cells show curved, bent, angulated forms of varying length, with variation in thickness of the individual bacillary body. Bacilli lying adjacent to vacuoles are also commonly curved about them.

It appears most likely that differences of appearance seen in bacilli in the chronic lepromatous lesion are partly determined by the development of the macrophage into the vacuolated cell with, at the same time, a marked diminution of the rate of growth of the bacilli. The organizing of the cells into a tissue is a factor in the shaping of the cells, which in turn affects the intracellular arrangements of the bacilli. The appearances of the chronic lesion suggest, even as do the clinical characters of leprosy, that in many cases the activity of the lesion is essentially stationary over long periods. The silvered preparations suggest that this is exactly the case, with only a slight degree of reproduction taking place in the highly vacuolated cell. The conception of the vacuolated



Figs. 5 to 8.—Development of the globus. Figure 5 shows highly vacuolated cells (well filled with bacilli) sharply set apart by the organization of the lesion. There is some coalescence of vacuoles. Such cells are forerunners of globi but do not necessarily develop into them. Figure 6 shows a well developed but young globus encased by a giant cell, bacilli well silvered throughout and moderate coalescence of vacuoles. Figure 7 shows a still older globus with weak silvering of bacilli, and figure 8 shows an old globus with complete coalescence of vacuoles and faintly silvered bacilli. Note, however, that there are well silvered bacilli in the encapsulating giant cell.

cell as a characteristic cell of leprosy is valid, but it must be recognized as a later result of intracellular growth. Vacuoles develop as the reproductive activity of the bacilli diminishes.

THE GLOBUS

The globus is a mass of bacilli, round or oval and sharply outlined. When large, it is often surrounded by a thinned-out giant cell, which may be so thin as to appear as a ring about the globus. The exact nature and significance of these structures and their manner of development have been much disputed, and the term is too frequently used to signify any large accumulation of bacilli in leprous tissues. From silvered preparations it is seen that the globus is indeed a characteristic structure following a definite evolutionary course.

In the fresh active lesions the structures commonly described under the heading of globi seem not to merit the term. There is no suggestion that the streams of actively growing organisms are developing into globi. In such cells the ordinary course is that in which vacuoles develop among groups of bacilli. The term "seed globi" applied to these groups of organisms by Cowdry⁵ does not appear justified.

From the silver preparations, the globus appears to arise within a cell, or a group of cells fused together, after the cell has undergone degenerative changes with loss of nucleus and cytoplasm. There is always, in the bacillus-laden cell, much lipid material, which persists in the globus, as do also the vacuoles, so that the early or young globus presents much the appearance of the parent cell. Larger or older globi show fusion of the vacuoles, and the aged globus shows a single vacuole occupying most of the globus, with the bacilli distributed largely about the margin. In small young globi bacilli are routinely found well silvered. In old globi bacilli become progressively more weakly silvered. Bacilli near the rim of the globus frequently are better silvered than those more centrally placed. Sections of leprous tissues containing globi stained by fuchsin show, on the other hand, the bacilli to be equally stained throughout the globus.

The globus frequently becomes something of a foreign body. In a definite small percentage of cases of leprosy there is formation of giant cells throughout the lesions. Many of these are of the foreign body type, and they routinely enclose globi. In regressive leprous lesions the globus is seen to be a highly persistent structure, usually with some form of multinuclear cell response to it. From the silvered sections it is seen that many bacilli in globi lose the affinity for silver, suggesting an alteration of their chemical structure. This is interpreted as a degenerative change, although the bacillus is preserved morphologically.

5. Cowdry, E. V.: *Am. J. Path.* **16**:1940, 103.

Globi are by no means uniformly observed in leprous lesions, and circumstances leading to their formation appear to be somewhat accidental therefore. The extremely large globi rarely observed seem to arise from coalescence of many cells, not from enlargement of the formed globus. The density of organisms in the globus naturally corresponds with the density of bacilli in the cells from which the globus arose.

DEGENERATIVE FORMS OF BACILLI

Weakly silvered and nonsilvered bacilli are readily identified in globi. Similar bacilli are also commonly encountered in minor numbers in almost every chronic lesion. They are seen in areas in which well silvered bacilli also occur, with all degrees of silvering observable. Both silvered and nonsilvered bacillary fragments are also seen. It seems clear that these are degenerate forms of the organisms. They may be fairly numerous in small areas, and their presence supports the idea that in the chronic leproma death and degeneration of bacilli are constantly taking place in some areas, even though other areas of the same lesion show evidences of progression. This is readily observed in large solid lepromas, in which the deeper areas show regression, while the most superficial ones show progression. It is suggested that well silvered organisms are healthy organisms; poorly silvered ones, degenerate or dead organisms. Obviously, such a conclusion can be drawn only on a broad basis from those specimens in which many degrees of silvering are observed in the same section. Unless absolute uniformity of technic is maintained, there will be differences in the degree of silvering in different blocks.

Pleomorphism of the lepra bacillus is marked in the chronic lesions but barely noticeable in the fresh lesions. It is especially prominent in areas in which degenerate forms occur, and is regarded as being related to the regressive phase of leprosy, not to the growth phase of the organisms. Study of such organisms has, in the past, led to erroneous conclusions with regard to the essential morphologic aspects of the lepra bacillus.

HALOS SURROUNDING BACILLI

In silvered preparations a halo is routinely seen about the bacillus as a narrow clear space investing the organism. It appears not to be a phenomenon of spherical aberration. However, halos are also encountered in fuchsin-stained sections and may be observed in sections of lesions containing bacteria quite unrelated to leprosy, so that their significance seems small. It is important to stress that the halos do not fuse with the vacuoles in the cells and that they are not to be regarded as such.

SUMMARY AND CONCLUSIONS

Leptra bacilli are readily silvered in blocks of lepromatous tissues with Jahnell's and other methods.

Bacilli silvered in the block undergo a minimum of distortion and yield a true picture of the infection of the tissues, scarcely obtainable with other methods. While such silvering is not practical as a routine clinical-pathologic procedure, it is highly valuable in the study of the lepromatous lesion.

Study of silvered sections shows that past concepts of the morphologic aspects and arrangements of bacilli are open to considerable revision. Actively growing organisms resemble minute colonies of fungi, and do not appear as "bundles" or "cigar packs." A new concept of the nature of the globus is offered.

Different degrees of silvering observed indicate that the weakly silvered bacillus constitutes one form of the degenerating organism.

SPLENIC CYSTS

H. T. TAMAKI, M.D.
NORRISTOWN, PA.

A PERUSAL of the medical literature on splenic cysts discloses that they are rather uncommon. The fact that they are rare can be attested to by the fact that not a single case of splenic cyst has been recorded in the surgical file at Jefferson Medical College Hospital since 1915. During a thirty year period from 1904 to 1934, Pemberton reported, only 4 cases of cysts of the spleen have been seen at the Mayo Clinic¹ in approximately 800 splenectomies. Denneen² reported that at the Bellevue Hospital in New York they were able to find record of but 8 cases of splenic cysts, none of which were of the hemorrhagic type, and that no cases were recorded at St. Vincent's Hospital during the thirty year period prior to 1940.

Reports of cases of cystic disease of the spleen in the American literature have been extensively reviewed by Fowler.³ According to Fowler, the first case of cystic tumor of the spleen was reported by Andral in 1829. Fowler,^{3b} in 1912, collected 89 cases of nonparasitic cysts of the spleen. In recent years Frank⁴ has written extensively on this subject and brought the total number of cases reported up to 1930 to 111. Other recent reviews on the subject have been written by Benton,⁵ Sherwin⁶ and Shawan.⁷

The total number of nonparasitic cysts reported up to 1939 was 137, according to Fowler's most recent review.^{3a} By 1942 McClure and Altemeier⁸ raised the total to 149. Gallagher and Mossberger⁹ described the one hundred and fifty-fifth nonparasitic cyst, and by the end of 1944 Harmer and Chalmers¹⁰ had increased the reported cases

From the Department of Pathology, Montgomery Hospital.

1. Roberson, F.: *Ann. Surg.* **111**:848, 1940.
2. Denneen, E. V.: *Ann. Surg.* **116**:103, 1942.
3. Fowler, R. H.: (a) *Internat. Abstr. Surg.* **70**:213, 1940; (b) *Ann. Surg.* **57**:658, 1913; (c) **74**:20, 1921; (d) **80**:58, 1924.
4. Frank, L. W.: *South. M. J.* **23**:212, 1930.
5. Benton, R. W.: *J. A. M. A.* **99**:1674, 1932.
6. Sherwin, B.; Brown, C. R., and Liber, A. F.: *Ann. Surg.* **109**:615, 1939.
7. Shawan, H. K.: *Arch. Surg.* **27**:63, 1933.
8. McClure, R. D., and Altemeier, W. A.: *Ann. Surg.* **116**:98, 1942.
9. Gallagher, P., and Mossberger, J. I.: *Ann. Surg.* **116**:933, 1942.
10. Harmer, M., and Chalmers, J. A.: *Brit. M. J.* **1**:521, 1946.

to 163. Since then at least 4 other cases have been reported¹¹ in the American literature, which would make the case I report the one hundred and sixty-eighth.

REPORT OF CASE

E. B., a 15 year old white boy, a student, was admitted to Montgomery Hospital on Oct. 6, 1948. He was referred to the hospital by the school physician, who noticed a large abdominal mass in the left upper quadrant on routine physical examination. The patient complained of nothing other than an occasional slight "pulling sensation" over the area of the mass. He first noted a slight enlargement just beneath the margin of the left rib approximately four years before, following an accident in which he fell from a tree, injuring his abdomen and sustaining a fracture of the left wrist. At that time the possibility of a ruptured spleen was entertained, and the surgeon introduced a needle into the peritoneal cavity, but obtained no blood. The mass remained small until three months prior to admission, when there was a gradual and fairly rapid increase in its size.

Examination disclosed a well developed, well nourished white youngster in no apparent distress. The head and neck appeared normal. A large mass was palpable in the left upper quadrant of the abdomen, which had produced a flaring of the lower ribs on the left side. The mass was easily visible and extended approximately 14 cm. below the costal margin. A peculiar resilience was noted on palpation. No definite fluid wave could be elicited, and the mass moved slightly with respiration. The rest of the examination disclosed nothing remarkable. Temperature, pulse and respiration were normal.

Urinalysis revealed a trace of albumin and occasional leukocytes. The hemoglobin content was 88 per cent; the red blood cell count was 4,530,000; the white blood cell count, 6,300, with 71 per cent polymorphonuclear leukocytes, 28 per cent lymphocytes and 1 per cent eosinophilic granulocytes.

The usual roentgen survey films of the chest and abdomen showed the lung fields clear, the cardiac shadow normal in size and configuration, and a definite elevation and splinting of the left leaf of the diaphragm. The roentgenogram of the abdomen showed a spherical mass in the left upper quadrant, with a rather sharply defined inferior border, which extended to the level of the body of the fourth lumbar vertebra. There was a curvilinear shadow of a calcified region extending transversely across the mass some 2 inches (5 cm.) above its inferior border. The stomach contained sufficient gas to demonstrate that it was displaced toward the right side, while the splenic flexure of the colon was displaced downward. The right kidney was normal in size, shape and position, while the left was rotated on its long axis and displaced downward, with both poles lying in the same horizontal plane. These findings were corroborated by an intravenous pyelogram and a roentgen study of a barium sulfate enema (Dr. R. D. Campbell).

Operative Note (Dr. D. M. Headings).—A long subcostal incision was made, the left rectus muscle was cut transversely and the peritoneum was opened. A large splenic cyst was discovered, which was mobilized and posterior fibrous adhesions were severed. The entire mass was enucleated in an intact state. The patient left the operating room in good condition. He had an uneventful course post-operatively and was discharged on the twelfth day.

11. (a) Jameson, E. M., and Smith, O. F.: U. S. Nav. M. Bull. **45**:537, 1945. (b) Neidhardt, H. W.: J. Kansas M. Soc. **45**:382, 1944. (c) Duggan, H. E.: J. Canad. M. Serv. **2**:283, 1945. (d) Duby, H.: New England J. Med. **237**:731, 1947.

Pathologic Report.—The specimen consisted of a large ovoid cyst, measuring 20 by 23 by 14 cm. and weighing 3,300 Gm. It was received intact. It presented in the main a dark purplish red surface, owing to the thinned-out condition of the normal splenic tissue over the cyst. The portion of the cyst which was posterior presented a thin, grayish red wall with no surrounding splenic tissue. This area also harbored numerous fibrous adhesions. At both poles there was a margin of grossly normal spleen. At one end it presented a narrow shelf 1.5 cm. in thickness and stretching out for a distance of approximately 8 cm. At the other pole there was a flat pyramid-shaped portion of the spleen, measuring 15 by 5 by 3 cm. The lumen of the cyst was filled with approximately 2.5 liters of dark brown, opalescent fluid. Examination of this fluid disclosed numerous red cells and many cholesterol crystals and necrotic debris. After the fluid content had been emptied out, numerous soft, light to dark gray nodules, varying in size from 1 to 5 cm. in longest dimension, were seen loose within the lumen (fig. 2). They were extremely soft and were apparently necrotic coagulum. The cyst wall was translucent where it was not covered by splenic tissue. In the main, however, it was surrounded by a layer of splenic tissue. The inner aspect of the cyst presented a light gray surface with a considerable degree of trabeculation. There was necrotic fibrinous material adherent to portions of the wall.

Section of the cyst wall disclosed a thick laminated hyalinized fibrous tissue. It was lined in part with a single layer of endothelium-like cells. There were areas, however, where the lining epithelium was not discernible. Within the wall were a few scattered foci of calcification. On the external aspect of the cyst wall there was a layer of compressed splenic tissue. There was moderate distortion of the normal splenic structure, also marked diminution of the number and size of the malpighian corpuscles. The surrounding reticulum, fibers and pulp were compressed and in many areas replaced by fibrous tissue with some degree of hyalinization. The infiltrate was made up primarily of lymphocytes along with numerous polymorphonuclear leukocytes. Eosinophilic granulocytes were quite prominent. There were a few foci of hemorrhage within the stroma. The splenic capsule disclosed a slight degree of hyaline change.

The sections taken through the necrotic material within the lumen disclosed a homogeneous light pinkish-staining material harboring countless cholesterol crystals with a sprinkling of polymorphonuclear leukocytes.

Diagnosis.—Hemorrhagic cyst of the spleen.

INCIDENCE

Various classifications of splenic cysts have been suggested in the literature. Such cysts have been classified according to morphologic aspects, pathogenesis, cell lining and contents; but each classification has its faults and limitations. The simplest classification would be to divide all splenic cysts into parasitic and nonparasitic groups. According to Fowler,^{3a} echinococcus cyst occurs about twice as often as nonparasitic cysts. The nonparasitic cysts may be further subdivided into primary and secondary cysts. Of the nonparasitic cysts, secondary cysts are approximately four times as common as primary cysts, as evidenced by Fowler's study of a large series of cases in which he found that 21 per cent were true cysts and 79 per cent false cysts.

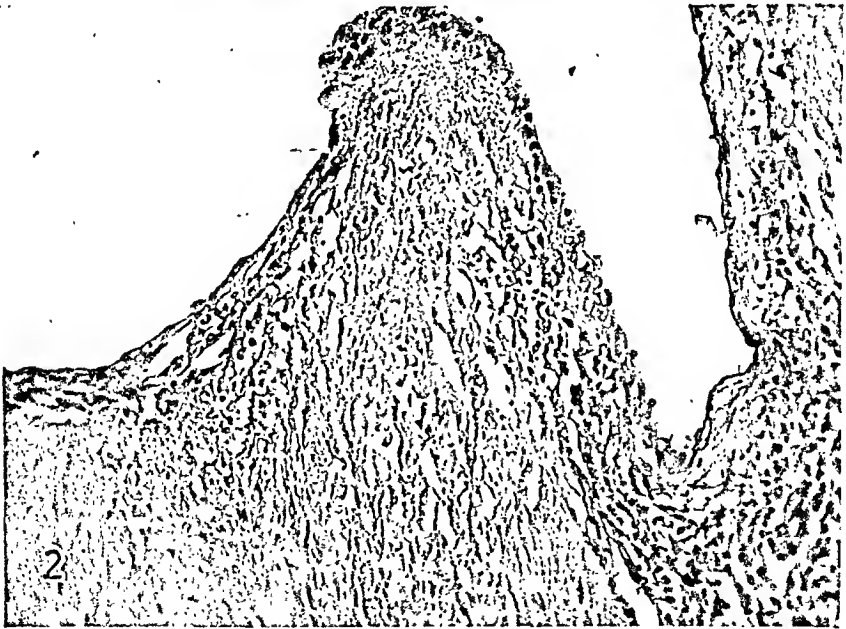
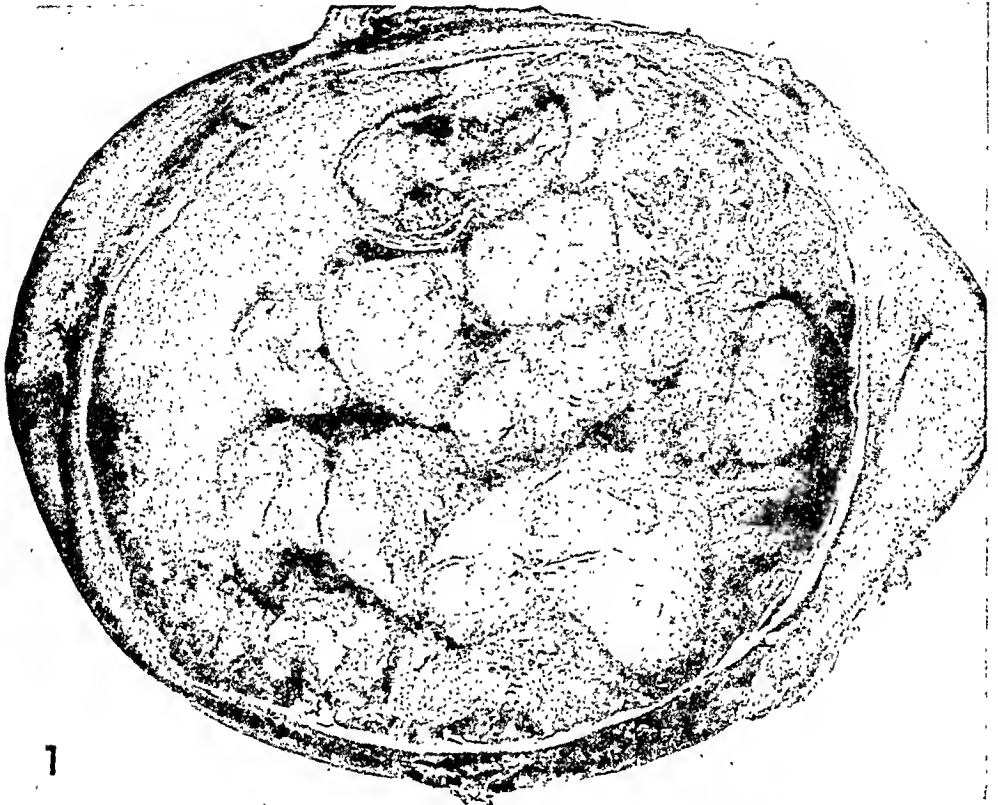


Fig. 1.—Large cyst of the spleen of a 15 year old boy, containing nodules (apparently necrotic coagulum).

Fig. 2.—Photomicrograph of the wall showing endothelium-like lining.

It is the purpose to confine the discussion primarily to the secondary or false cysts, which are of the greatest clinical importance. These secondary or false cysts may be either single or multiple. In nearly 80 per cent of the cases of nonparasitic cyst a single cyst is found. It may be subcapsular or situated deeply within the organ, the former type being nearly twice as common. Such cysts vary considerably in size; one may measure as little as a few centimeters in diameter, and another may fill the greater part of the abdominal cavity. Hamilton and Boyer¹² reported a hemorrhagic splenic cyst which contained 7 liters of fluid, and Heurtaux¹³ described one containing 10 liters of fluid. Few of the reported solitary nonparasitic cysts were calcified. Snoke,¹⁴ in 1943, was able to find but 7 cases of solitary calcified cysts of the spleen described in the literature. Since then Neidhardt^{11b} and Duggan^{11c} have reported 2 more cases, making the present case the tenth case of calcified solitary false cyst. Secondary cysts of the spleen may be found at any age. The majority fall between the ages of 20 and 40, and 65 per cent are said to occur in females.

CAUSES

In the consideration of the etiologic factors of splenic cysts it is agreed by all that trauma is important. In the reviews of others, as well as in a review of the more recently reported cases, a history of trauma can be obtained in nearly one fourth of the cases. The interval between the injury and the development of the cyst was variable, although in fully 50 per cent of the cases the cyst occurred within three years and in nearly 90 per cent within ten years.^{3a} The trauma was usually a fall on the abdomen, as in our case, or a direct blow over the splenic area.

Of the cysts with history of trauma, nearly 80 per cent were of the hemorrhagic type. McClure and Altemeier⁸ suggested that they are actually encysted hematomas, which result from an injury which fails to produce laceration of the surface of the spleen but which tears deep-seated vessels and causes hemorrhage. In time a cyst is formed with a bloody fluid content surrounded with a fibrous wall. It is also possible for intrasplenic hemorrhage to occur spontaneously, without any antecedent history of trauma.

Menstruation and pregnancy appear to play some part in the formation of splenic cysts. Of the cysts occurring in women, the majority were found during the child-bearing age. They have been described in women with menstrual irregularities as well as in pregnant women. According

12. Hamilton, C. S., and Boyer, E. H.: *Ann. Surg.* **73**:58, 1921.

13. Heurtaux, A.: *Bull. et mém. Soc. de chir. de Paris* **24**:928, 1898.

14. Snoke, P. O.: *Am. J. M. Sc.* **206**:726, 1943.

to Mannier (quoted by Frank⁴), the instance in which such a cyst occurs during the reproductive period is explained by the fact that the spleen becomes congested in pregnancy and during menstruation. In support of this theory, Frank⁴ described a case in which a large cyst was marsupialized, and blood was discharged from it at each menstrual period after the operation. This, however, is not convincing proof of a direct relationship, as there is some doubt as to the origin and the nature of the cyst described. There are other reports of cases in the literature to suggest that the cyst of the spleen is related to menstruation, such as those in which metrorrhagia was relieved after splenectomy.¹⁵ The unconvincing evidence given would lead one to question a direct relationship of menstruation or pregnancy and formation of a splenic cyst. As Fowler suggested, congestion during menstruation may result in an increase in size of a preexisting cyst. Infarctions of the spleen may possibly be related to pregnancy with resulting secondary hemorrhage and cyst formation. Frank⁴ expressed the belief that splenic cysts are all serous in type in the beginning but that some become blood cysts because of the bleeding into the cyst which may occur as the result of splenic congestion associated with menstruation.

The possibility that the splenic cyst is related to some antecedent disease has been discussed by some writers. Malaria is mentioned most frequently as a predisposing cause. According to Harmer and Chalmers,¹⁰ splenic cyst is not uncommon in West African natives, in whom malaria is almost universal. Other less frequently associated diseases are syphilis, typhoid, paratyphoid and mumps. In all probability the relationship is perhaps indirect, in that the splenomegaly associated with these diseases would more readily subject the organ to trauma.

Embolism or thrombosis of the splenic vessels has been shown to cause cyst formation, and this type has been described recently by Dabrzaniecki.¹⁵ Cysts may also occur within the splenic parenchyma as a result of endarteritis.

Snyder and Rezek¹⁶ favored the theory of malformation, basing their opinion on the fact that congenital malformations may become manifest later in life through postembryonic influences.

PATHOLOGIC ASPECTS AND GENESIS

Large splenic cysts may be lymphogenous or hemorrhagic in origin. The contents, therefore, may be serous, serosanguineous or purely hemorrhagic in nature. The blood may be coagulated, black to brown and frequently possessed of a sparkling sheen due to the presence of

15. Dabrzaniecki, W.: *Ann. Surg.* 92:67, 1930.

16. Snyder, J. W., and Rezek, P. R.: *South. M. J.* 36:263, 1943.

cholesterol crystals. There is considerable variation in the thickness of the cyst wall. Some older cysts may lack a true wall. The inner surface may be smooth, rough or trabeculated. The wall itself is made up essentially of dense connective tissue, which may in some cases harbor foci of calcification. Approximately 9 per cent of the reported cysts were calcified.² Surrounding the cyst wall there may be a stretched-out layer of splenic tissue of varying thickness. Depending on the degree of intracystic pressure, the structure of the splenic tissue may or may not be distorted. In the area of great pressure there may be signs of atrophy with considerable compression of the reticulum fibers and pulp and decrease in size and number of the lymph follicles.

Microscopic study of the solitary hemorrhagic cysts discloses a laminated dense hyalinized connective tissue wall which may or may not present a cellular lining. Although secondary or false cysts are said not to possess a true epithelial lining, a single layer of endothelial cells or even a stratified squamous epithelium has been described in numerous reports.¹⁷ The nature of the lining cells depends on different factors, such as cause, size and age of the cyst. It is conceivable that serous cysts may arise from lymphatic vessels; or, if the presence of lymphatic channels in the spleen be denied, the endothelial lining could be derived from the endothelial cells of the spleen or possibly from splenic inclusions of the investing peritoneal endothelium. Even in a primary blood cyst an endothelial lining may be acquired,^{17a} as demonstrated in my case. In all probability the squamous cell lining described resulted from the metaplasia of endothelial cells.

DIAGNOSIS

In many instances the patient with a splenic cyst is asymptomatic, particularly if the cyst is small. Not infrequently, however, as was observed in my case, even a large cyst may cause minimal or no symptoms at all. Symptoms of pressure are the usual complaints with the large cysts. They may express themselves as a feeling of heaviness or a dragging sort of pain. There may be dyspepsia or inability to eat a large meal, owing to the compression of the stomach and intestines. More rarely, dyspnea, constipation or diarrhea may be present.

Although Sweet¹⁸ stated that "large cysts present a clinical picture which is so characteristic that the diagnosis can be readily made," the diagnosis of splenic cyst was rarely made in the reported cases. In the present case the preoperative diagnosis of splenic cyst was made by some of the physicians who saw the patient in consultation.

17. (a) Paul, M.: *Brit. J. Surg.* 30:336, 1943. (b) Shawan.^{7c} Jameson and Smith.^{11a}

18. Sweet, R. H.: *New England J. Med.* 228:705, 1943.

Perhaps the greatest help in diagnosis can be had from careful physical and roentgen examinations. Palpation of the abdomen may disclose a tumor in the left upper quadrant of the abdomen. If the cyst is large, the costal margin of the overlying left rib is usually pushed outward. This is not a usual finding with cyst of the pancreas or the ovary. At times the cystic nature of the tumor may be suspected from the doughy sensation. The roentgen examination may be the determining factor in many cases.¹⁹ A flat roentgen plate of the abdomen may disclose that the left side of the diaphragm is high, and the motion may be seen to be impaired when observed by fluoroscope. Occupying the left upper quadrant may be a large soft tissue mass either with or without foci of calcification. In practically all reported cases of large cyst of the spleen the roentgenograms show the stomach to be pushed to the right and backward and the splenic flexure to be displaced downward and to the right. Occasionally there may be some displacement of the left kidney, usually downward. Although the renal pelvis is not usually altered, there may be some distortion due to the downward displacement. Obliteration of the psoas muscle on the left side is also described. It is interesting to note that within four weeks after splenectomy in cases of splenic cyst the displaced organs are back in their normal position.

DIFFERENTIAL DIAGNOSIS

In considering the differential diagnosis of cyst of the spleen, one must consider cyst of the kidney, the ovary, the pancreas, the mesentery or the omentum, as well as cyst of the left lobe of the liver. Other causes of splenomegaly must also be considered along with the rare retroperitoneal tumor.

Of some help in the diagnosis of splenic cyst is the observation that it is usually more superficial than one would ordinarily find a renal tumor to be. The pancreatic cyst, as well as the ovarian cyst, occupies a lower position in the abdomen. Sweet¹⁸ stated that the outward flaring of the left costal margin does not occur as a rule in the case of pancreatic cyst and rarely in that of ovarian cyst. Ostro and Makover²⁰ described a case of chronic myelogenous leukemia with marked splenomegaly, in which the spleen extended into the pelvis and to the midline. As contrasted with their roentgenograms of splenic cyst, those made in a case of leukemia disclosed that the left side of the diaphragm was in normal position, with no spreading of the ribs, no displacement of the kidney and no obliteration of the psoas muscle. Furthermore, although the descending colon was displaced to the right, the splenic flexure, as well as the cardiac end of the stomach, was in normal position.

19. Elkeles, A., and James, J. I. P.: *Brit. J. Radiol.* **16**:59, 1943.

20. Ostro, M., and Makover, H. B.: *Am. J. Roentgenol.* **37**:782, 1937.

TREATMENT

There is but one method of choice for the treatment of splenic cysts. Except in those instances in which adhesions may make it inadvisable, splenectomy should be performed in all cases of large cyst of the spleen. Over an extensive series of cases a mortality rate of approximately 4 per cent was reported for cases in which splenectomy was done.^{3a} Various other methods of treatment have been tried—marsupialization, puncture of the cyst, coagulation of the contents and extirpation of the cyst—but the results have been generally unsatisfactory.⁵

SUMMARY

A review of the literature discloses reports of 167 cases of splenic cyst. An additional case is reported. The etiologic and pathologic aspects, as well as the diagnosis and treatment, of cysts of the spleen are discussed.

EFFECTS OF FOLIC ACID DEFICIENCY AND A FOLIC ACID ANTAGONIST ON CHICKS

EPHRAIM WOLL, M.D.
BURLINGTON, VT.

THE isolation, the synthesis and the clinical application of folic acid and its derivatives opened new roads to the study of nutrition, cellular metabolism and certain aspects of tumors. General reviews of the subject have been made by Jukes and Stockstad¹ and Sargent.² Recent studies of folic acid deficiency have been reported by Cartwright and associates³ and Rinehart and Greenberg.⁴ The first clinical application of folic acid conjugates and antagonists in the treatment of cancer was made by Farber and co-workers.⁵ The effects of folic acid, folic acid deficiency and folic acid antagonists on Rous sarcoma were described by Little and associates⁶ and Woll.⁷

In the latter study pathologic changes were seen in the deficient and the antagonist-treated animals. The purpose of the experiments to be described was to expand these observations and to learn the sequence of events in terms of morphology.

MATERIALS AND METHODS

Approximately 200 1 day old New Hampshire Red chicks were used. The choice of very young birds was guided by a desire to have rapidly growing subjects in which nutritional disturbances may manifest themselves sharply. All

From the Research Department, Lederle Laboratories Division, American Cyanamid Company, Pearl River, N. Y., and the Pathology Department, Children's Medical Center, Boston.

1. Jukes, T. H., and Stockstad, E. L. R.: *Physiol. Rev.* **28**:5, 1948.
2. Sargent, F., II: *New England J. Med.* **237**:667 and 703, 1947.
3. Cartwright, G. E.; Fay, J.; Tatting, B., and Wintrobe, M. M.: *J. Lab. & Clin. Med.* **33**:397, 1948.
4. Rinehart, J. F., and Greenberg, J. D.: *Am. J. Path.* **24**:710, 1948.
5. Farber, S.; Cutler, E. C.; Hawkins, J. W.; Harrison, J. H.; Peirce, E. S., II, and Lenz, G. G.: *Science* **106**:619, 1947. Farber, S.; Diamond, L. K.; Mercer, R. D.; Sylvester, R. F., Jr., and Wolff, J. A.: *New England J. Med.* **238**:787, 1948.
6. (a) Little, P. A.; Sampath, A.; Paganelli, V.; Locke, E., and Subbarow, Y.: *Tr. New York Acad. Sc.* **10**:68, 1948. (b) Little, P. A.; Sampath, A., and Subbarow, Y.: *J. Lab. & Clin. Med.* **33**:374, 1948.
7. Woll, E.: *Tr. New York Acad. Sc.* **10**:83, 1948.

the chicks received water, a diet and certain compounds to be described. They were divided equally into the following dietary groups:

Group 1 (control)—a commercial chick feed.

Group 2—a synthetic folic acid-free diet developed by Dr. J. J. Oleson, of our laboratories.

Its composition is as follows:

Cerelose (dextrose monohydrate).....	53.0%	Nicotinamide.....	3 mg. %
Alcohol-extracted casein.....	22.0%	Pyridoxine.....	0.5 mg. %
Salt mixture.....	4.3%	Thiamine hydrochloride.....	0.3 mg. %
Calcium gluconate.....	3.0%	Biotin.....	0.03 mg. %
Gelatin.....	8.0%	Riboflavin.....	0.5 mg. %
Ruflex (purified cellulose containing 70% alpha cellulose).....	4.0%	Inositol.....	100 mg. %
Soy-bean oil.....	5.0%	p-aminobenzoic acid.....	5 mg. %
Cholic acid.....	0.25%	Alpha tocopherol (vitamin E).....	5 mg. %
Cystine.....	0.45%	Vitamin K.....	0.2 mg. %
Choline chloride.....	200 mg. %	Vitamin A.....	3,500 units %
Calcium pantothenate.....	3 mg. %	Vitamin D.....	200 units %

Group 3—the synthetic diet plus a daily intraperitoneal injection of 100 micrograms of folic acid in aqueous solution.

Group 4—a commercial chick feed plus a daily intraperitoneal injection of 0.1 mg. of 4-aminopteroylaspartic acid.

Group 5—a commercial feed with 80 mg. of 4-aminopteroylaspartic acid mixed in each kilogram of feed.

Group 6—the synthetic folic acid-free diet for ten days, followed by the same diet plus a daily intraperitoneal injection of 0.1 mg. of folic acid for the next five days.

The doses of folic acid and antagonist were judged optimal on the basis of the extensive experience of Little and co-workers^{6a} in the study of nutritional control of the Rous sarcoma.

Three to 5 birds from each group were killed by decapitation on the second, third, fourth, fifth, seventh, tenth, twelfth, fifteenth, seventeenth and twentieth days of the experiment and examined immediately. Blocks of tissue were taken from all the viscera and from pectoral muscle, skin, tongue and tibial marrow. In a few cases blocks were removed also from the brain and the spinal cord. Numerous fixatives and stains were used. Fixation in formaldehyde-Zenker solution, paraffin embedding and hematoxylin-eosin staining were found to be a most useful procedure. In certain cases, iron, fat and connective tissue stains were of special value. Care was taken to obtain areas that would be anatomically comparable as to location and as to plane of section. This was particularly important in the study of the marrow, which was removed by snipping off the proximal epiphysis and then, by gentle pressure and traction, obtaining a fairly intact and uniform pencil of tissue. With the older birds, longitudinal splitting of the bone was required. No decalcification of the marrow block was necessary. Animals which died during the experiment were generally not examined, as it was considered that the histologic appearances would be unreliable.

In order to learn the effects of malnutrition in general, 20 chicks were given a commercial feed but in much reduced amounts. Thus the birds which weighed 35 to 40 Gm. on the first day of the experiment weighed only 30 Gm. at the end of ten days, compared with 55 to 60 Gm. for the controls. This group was killed and examined in the same way as the others.

An additional group of 10 birds received a total of 200 micrograms of 4-aminopteroylaspartic acid in two days by intraperitoneal injection. These were killed on the third day and treated similarly to the other groups.

GROSS OBSERVATIONS

Group 1 (control).—There were no abnormalities of development or of nutrition, nor was there evidence of spontaneous disease.

Group 2 (folic acid-free diet).—The chief findings were progressive emaciation and retarded development of the body and the viscera. The development of the body and the viscera was estimated to be 50 to 60 per cent of the normal on the fifteenth to twentieth days. There was no appreciable delay in the appearance of the wing feathers or the comb. The blood was watery and pale red. The marrow, which was at first pale red and more moist than normal, became light brown and viscid. The bowel, especially the distal third, including the ceca, was often but not always markedly distended with foul-smelling, abnormally watery feces. Grossly there was no appreciable increase in its fat content. The yolk sac was not any smaller than those in comparable controls. The gallbladder was sometimes distended with dark green, viscid bile.

Group 3 (folic acid-free diet plus folic acid by injection).—There were no grossly appreciable differences from the control birds.

Group 4 (regular diet with 4-aminopteroylaspartic acid in the diet).—The changes were similar in all respects to those obtained with the folic acid-free diet. However, the lesions were less pronounced and appeared later. From the fifteenth day on, several birds appeared to have recovered and to be almost normal except for a slightly smaller size of body and viscera.

Group 5 (regular diet plus 4-aminopteroylaspartic acid by injection).—The changes were the same as in the folic acid-free group. Toxic signs developed more rapidly and led to the death of approximately 10 to 15 per cent of the birds before the twentieth day.

Group 6 (folic acid-free diet for ten days, followed by same diet supplemented with a daily intraperitoneal injection of 0.1 mg. of folic acid).—The findings to the tenth day were identical with those of the similar group already described. From the twelfth day, i. e., after the feeding had been supplemented parenterally with folic acid, there was a rapid and marked improvement in nutrition and development. However, by the fifteenth day recovery was not complete.

The starved animals were malnourished, anemic and underdeveloped. The yolk sac was much smaller in these than in the controls, while the gallbladder was often distended. The tibial marrow was pale, slightly viscid or watery but not light brown.

The animals which received a large amount of the antagonist in two days appeared stuporous before death. At autopsy no notable gross abnormalities were seen.

MICROSCOPIC OBSERVATIONS

General Considerations.—There is a rapid change in the gross and the microscopic anatomy of the chick in the first weeks of life. For example: Granulopoiesis, at first slight and seen only along the endosteal and the epiphysal margins, becomes pronounced in the first three weeks and permeates the whole marrow (fig. 1). Foci of small mononuclear cells, considered by some investigators to be thrombocytes, appear in the second and third week. The amount of fat in the liver decreases steadily. Little or no fat is present in the usual depots. Thus, to evaluate properly the pathologic condition seen, it is important to compare the treated birds with the normal controls from day to day and, as far as possible, to compare planes that are anatomically equivalent even from a microscopic standpoint.

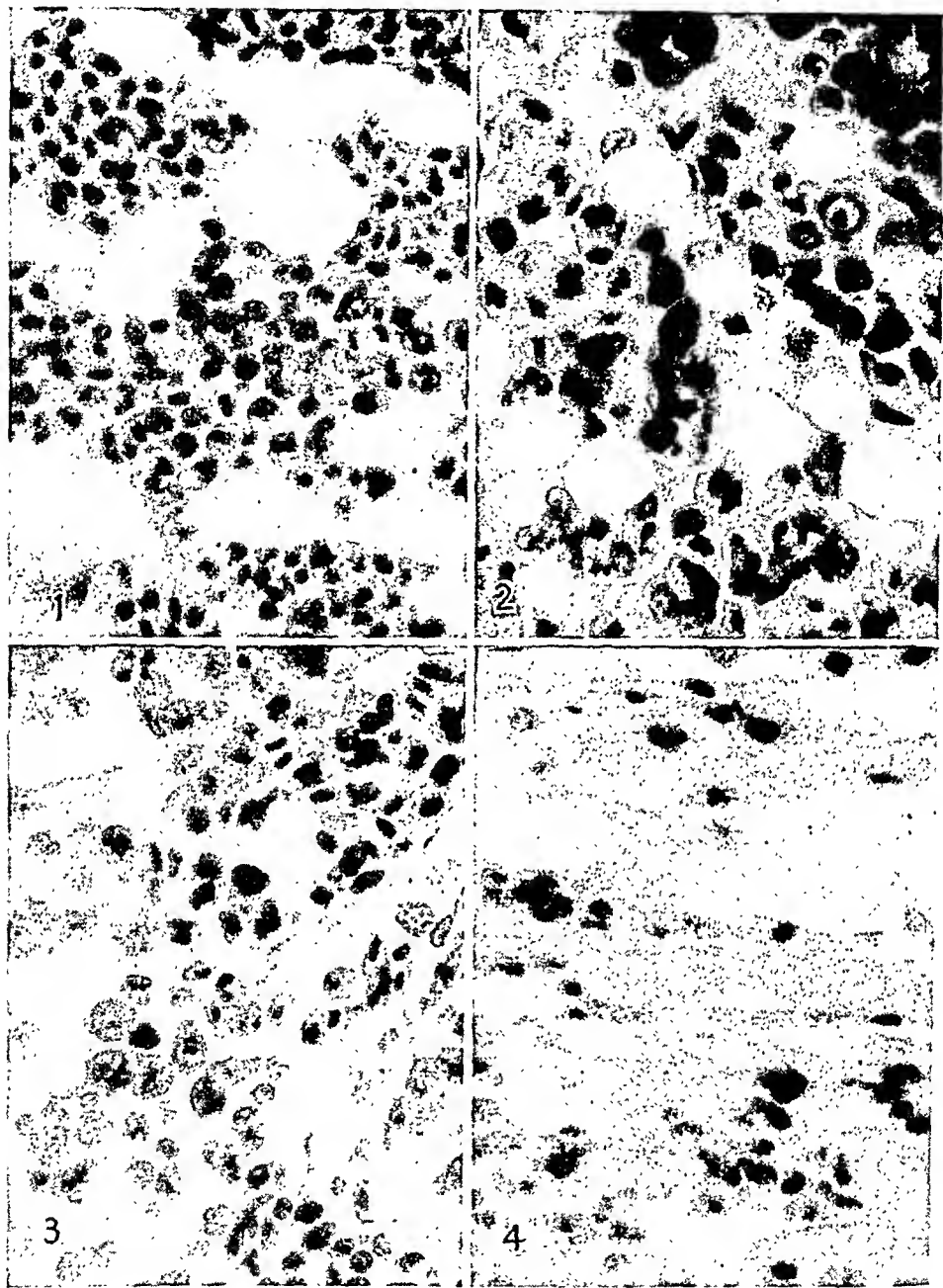


Fig. 1.—Marrow of a normal 15 day old chick. Note the proportion and the size of the various blood cell elements. The cells with deep-staining nuclei are erythrocytes. The white cells show cytoplasmic granules or rods. The blast cells have a homogeneous cytoplasm and a stippled nucleus. Hematoxylin and eosin; $\times 650$.

Fig. 2.—Marrow of a 15 day old chick that was restricted to a folic acid-free diet. Note the definite decrease in number of erythrocytes and the increase in size and number of immature red and white blood cells. Hematoxylin and eosin; $\times 650$.

Fig. 3.—Marrow of a 15 day old chick which was treated with 4-aminopteroyl-aspartic acid by parenteral injection. Note the severe pancytopenia. Hematoxylin and eosin; $\times 650$.

Fig. 4.—Marrow of a 17 day old chick restricted to a folic acid-free diet. The aplasia and the myxomatous character of the stroma are seen. Hematoxylin and eosin; $\times 650$.

The changes in the folic acid-deficient birds and those in the birds treated with the antagonist were found to be essentially the same. They will therefore be described together. The differences and similarities of these birds as compared with the malnourished group will be described later. The most impressive departures from normal were seen in the bone marrow and the bowel.

Bone Marrow.—As early as on the second day there was a sharp reduction of the number of mature erythrocytes. This apparently was due to a depression of their formation rather than to increased destruction. No evidences of the latter were found in the marrow or the viscera. On the third to seventh day there was a marked relative increase of the number and often of the size of immature red and white blood cells. This was especially notable of the blast cells. The normal rise in granulopoiesis somewhat masked the true picture. Nevertheless, the proportion of myelocytes was abnormally high (figs. 2 and 3). From the seventh to fifteenth day pancytopenia developed. It progressed into total aplasia (fig. 4). Past the fifteenth day severe aplasia was prevalent. In this stage, there was progressive reduction of the fat vacuoles of the stroma, and the connective tissue became a myxomatous, "snudged" ground substance. This was not seen in the fat elsewhere. Instead, the usual changes of inanition were observed. Macrophages containing numerous iron-staining granules were seen in the marrow, the spleen and the thymus but not in the liver. There was polychromasia of the young red blood cell but no anisocytosis or poikilocytosis. The wide range of the size of the normal fowl erythrocyte makes this judgment unreliable. Mitotic activity, particularly prominent in erythropoiesis, was not reduced except in advanced aplasia. There was no extramedullary hemopoiesis. The birds which received a folic acid-free diet supplemented with folic acid throughout the time of the experiment showed no abnormalities except for a moderately increased proportion of normal-appearing erythroblasts.

Bowel.—The earliest and most impressive departures from the normal were seen in the small bowel, especially in the distal third (fig. 5). On the third to fifth day the cells of the glands swelled. The nucleus became vesicular, the cytoplasm lighter staining and the cell margin indistinct. At times the cells appeared multinucleated. Mitotic activity was apparently not impaired (fig. 6). Somewhat later (five to ten days) the changes described were accentuated. The configuration of the cells at the base of the gland was greatly disturbed. The lumen of the gland was narrowed. From the tenth day on, numerous retention cysts, filled with a deeply staining mucoïd material, appeared. Still later there was atrophy of the mucosa, characterized by a narrowing and by an increase of the fibrous elements of the stroma (fig. 6). At no time was ulceration of the small and large bowel seen. When the aforementioned changes were most severe, the esophageal, colic and occasionally lingual mucosa but not the gastric (proventriculus and ventriculus), showed the same abnormalities, especially at the base. In 2 instances only were there frank erosion and ulceration of the mucosa of the crop. In these cases the mucosal changes were most severe.

Other Organs.—No appreciable abnormalities were seen in the mucosa of the respiratory, biliary, pancreatic, urinary or genital tracts. No significant changes were seen in the other viscera or the musculature. The skin and its appendages appeared normal throughout. This may be due to the short time of the experiment. Late in the experiment the thymus, the spleen and the lower part of the bowel had slightly less than the normal number of mature lymphocytes. In the thymus the corpuscles of Hassall were larger and more numerous than

progresses to a total aplasia with slight hemosiderosis; (*b*) degeneration of the glandular epithelium of the small and large bowel and, to a lesser extent, of the mucosa of the esophagus and the tongue, characterized by swelling of the cells and their nuclei, loss of normal cell configuration, gland retention cysts and ultimately atrophy.

Identical lesions are produced by a folic acid antagonist, such as 4-aminopteroylaspartic acid.

In the older animal the antagonist-induced deficiency is not so impressive.

The possible implications of the observations with respect to tropical sprue and Rous sarcoma are discussed.

University of Vermont College of Medicine.

General Reviews

SPONTANEOUS DEMYELINATING DISEASES OF ANIMALS

A Study in Comparative Pathology

LESTER S. KING, M.D.

AND

MARJORIE C. MEEHAN, M.D.

CHICAGO

A GREAT variety of spontaneous disorders of the nervous system exists in lower animals. Some of these have received considerable attention from both the descriptive and the experimental standpoint; to others but little attention has been paid. Apart from any economic advantage, the study of animal diseases is of value from a double standpoint—first, as disclosing fundamental pathologic relationships in natural hosts; second, as casting light on human disease. In general, pathologists have been especially interested from the latter standpoint.

It is the purpose of this communication to review in critical fashion certain spontaneous diseases of the nervous system in animals which are relevant to the problems of demyelination found in man and to discuss some of the general pathologic considerations raised thereby.

DEMYELINATION IN MONKEYS

A spontaneously occurring demyelinating disease of apes and monkeys has been reported repeatedly in the European literature of the present century. Although the descriptions of clinical symptoms and pathologic changes have varied in minor details, and the interpretations of the nature of the conditions have not all agreed, there seems little doubt that most or all of the reports refer to the same disease. Lesions may occur in the white matter of the hemispheres, the optic nerves and tracts, the spinal cord or in any combination of these.

Rothmann¹ in 1906 described a case which he called a "tabes-like disorder of apes." A rhesus monkey which had not been subjected to any experimental procedures was observed to have limited vision and slow, clumsy movements. Neurologic examination showed diminution of patellar reflexes, diminished sensation in the limbs and unsureness of movements. Despite these symptoms, the monkey was

From the Pathological Laboratory and Psychiatric Service, Illinois Masonic Hospital.

1. Rothmann, M.: *Monatschr. f. Psychiat.* 20:204, 1906.

used for experimental purposes. The decussation of the pyramids of the medulla oblongata was sectioned, and later the arm area of the cortex was extirpated bilaterally. The animal died three months after the first operation. Postmortem examination of the spinal cord and the optic chiasm—no mention is made of other parts of the brain—revealed an area of degeneration of the posterior columns varying somewhat in extent at different levels of the cord but most marked in the thoracic region. At all levels it was most noticeable around the posterior septum and in the peripheral (dorsal) portion of the columns. Except for demyelination, the changes were not clearly described. Demyelination was also discovered in the optic chiasm. Rothmann considered the condition strongly suggestive of tabes but was reluctant to identify it as tabes because of the difference in distribution of the lesions—most marked in the dorsal region. No later writer has accepted this disease as tabes.

In 1908 Schröder² reported an excellent clinical and pathologic study of a spontaneous disease in *Cercopithecus fuliginosus* which he thought might be an earlier stage of the condition described by Rothmann. In 1919 Kuhn and Steiner³ and Steiner⁴ described a monkey which had received by intra-abdominal injection 1 cc. of spinal fluid from a patient with multiple sclerosis. Eleven months later weakness of the hindlimbs was observed, but it cleared up quickly. Fifteen months after the injection, spastic paralysis appeared, and after four weeks' observation the animal was killed and examined. In the white matter of both hemispheres were many foci, some confluent, of demyelination, in most of which some axis-cylinders remained. Glial fibers were numerous, and at the periphery of the lesions there were many lipophages (*Körnchenzellen*) and much fat. Kuhn and Steiner considered the condition typical of multiple sclerosis, probably induced experimentally, but they admitted the possibility of spontaneous disease. Later writers have generally agreed that this case should be included among the spontaneous cases. Davison⁵ stated that Steiner himself later reached this conclusion. No further examples have been found in the literature until 1930. But since then more than 40 cases have been carefully studied in continental Europe and England, and reference has been made to many other animals sick with apparently the same symptoms.⁶ In 1944 Scherer⁷ published a thorough review of all reported cases, including 20 which he had personally studied.

2. Schröder, P.: Arch. f. Psychiat. **44**:193, 1908.

3. Kuhn, P., and Steiner, G.: München. med. Wchnschr. **66**:1245, 1919.

4. Steiner, G.: Ztschr. f. d. ges. Neurol. u. Psychiat. **17**:491, 1919.

5. Davison, G.: J. Neurol. & Psychopath **14**:227, 1933.

6. (a) Perdrau, J. R.: J. Path. & Bact. **33**:991, 1930. (b) Levaditi, C.; Lepine, P., and Schoen, R.: Compt. rend. Soc. de biol. **104**:986, 1930. (c)

Although the greatest number of affected animals have been rhesus monkeys, most primates seem to be susceptible, the disease having been reported observed in orang-utans, baboons, various types of monkey and a lemur. It has not been found in any of the platyrrhine monkeys, chimpanzees or gorillas. Sex is not a factor. The age distribution, obviously, is not known accurately, but most or all of the subjects were adults. Although not all the accounts mention the origin of the animals, none state that these were born in captivity. Several of the animals were known to have been captured in Sumatra; others in Simla. It is not known whether the disease occurs among animals in their native state, since all the studies have been made in zoos or scientific laboratories. The case described by Hurst and McLennon^{6a} occurred in an open-air zoo with conditions comparable to the natural environment.

The clinical picture is somewhat variable. Usually peculiarities of gait, clumsiness of movement and weakness of the limbs were the earliest signs, followed by increasing ataxia and partial paralysis. Some of these animals showed visual defects up to complete blinding. There is evidently much uncertainty whether sensory changes occur. In another group of animals, blindness was the first, and sometimes the only, symptom. In at least 1 case no clinical neurologic signs were observed; the animal died of tuberculosis, and the typical lesions of the brain were an unexpected finding in a routine autopsy.^{6m} The animals usually lived from one to six months after the onset of symptoms, and death often occurred from intercurrent disease, frequently tuberculosis.

Although most of the cases were carefully studied, there were no constant significant findings outside the nervous system. Only once was any abnormality of the meninges noted—when Gärtner^{6f} described a few lipophages in the pia mater overlying a lesion of the spinal cord. No changes have been discovered in any portions of the gray matter, cortex, basal ganglions or central gray matter of the cord. The lesions were confined to the white matter of the cerebral hemispheres, the

Schob, F.: *Ztschr. f. d. ges. Neurol. u. Psychiat.* **135**:95, 1931. (d) Scherer, H.: *ibid.* **141**:212, 1932. (e) Gärtner, W.: *Klin. Wchnschr.* **11**:905, 1932; (f) *Arch. f. Psychiat.* **99**:822, 1933. (g) Levaditi, C.; Hornus, G., and Schoen, R.: *Compt. rend Soc. de biol.* **113**:288, 1933; (h) *Bull. Acad. de méd., Paris* **110**:394 and (i) 406, 1933. (j) Hamerton, A. E.: *Proc. Zool. Soc., London* **104**:389, 1934; (k) **106**:659, 1936; (l) **107**:443, 1937. (m) Scherer, H. J.: *Rev. neurol.* **68**:897, 1937. (n) Hamerton, A. E.: *Proc. Zool. Soc., London*, s.B **108**:489, 1938; (o) **111**:151, 1941. (p) Von Bogaert, L.: *Ann. Inst. Pasteur* **63**:315, 1939. (q) Hurst, E. W., and McLennon, G. C.: *M. J. Australia* **2**:661, 1941. Davison.⁵

7. Scherer, H. J.: *Vergleichende Pathologie des Nervensystem der Säugetiere*, Leipzig, Georg Thieme, 1944.

optic nerves and tracts and the posterior and lateral columns of the spinal cord. The cerebellum and the corpus callosum were never involved. In the cerebrum there was nearly always sparing of the U fibers. Although in the majority of cases lesions occurred in all three of the typical locations—hemispheres, optic nerves and spinal cord—in several cases the cord was spared, while in others it was either not examined or not described. Again, many reports lacked descriptions of the optic system. The hemispheres were evidently normal in Rothmann's case and in some later cases.

In the hemispheres the lesions consisted of bilaterally symmetric areas of demyelination which in some cases were disseminated and in others diffuse. Authors describing widespread, or diffuse, areas generally agreed that they were made up by confluence of smaller foci. The foci might be round or irregular in shape. They were usually most numerous centrally and smaller and less numerous near the cortex, not involving the U fibers, except in a few instances. The lesions were most frequent in the parietal lobes, but common in the frontal and the occipital. Scherer⁷ expressed the belief that the disease usually started in the parietal lobes and extended gradually forward and backward to the frontal and occipital areas. No foci have been described in the temporal lobes or orbital convolutions. Scherer stated that they were common in the upper third of the internal capsule. No plaques were found in the corpus callosum, but secondary degeneration of its fibers may be observed. Many of the lesions appeared to be perivascular. Demyelination was usually complete near the center of the foci, but sometimes there was a surrounding area of partial demyelination, shown by pallor in myelin stains. There is some difference of opinion as to the relative preservation of axis-cylinders, which usually appeared diminished in number and frequently damaged. Scherer stated that in the oldest lesions the axis-cylinders appeared better preserved than the myelin sheaths. In the earliest lesions there was some macroglial proliferation, followed later by microglial increase and the appearance of many lipophages. Except in the oldest lesions, there was usually much fat. "Gemästete glia cells" were frequent, especially around the vessels. In a few instances some perivascular round cell infiltration has been noted, but this was uncommon and seen only in older lesions. The vessels within the lesions showed no significant changes except some dilatation.

Lesions in the optic nerves, tracts and chiasm were frequent, but the descriptions are variable. Scherer⁷ found 4 cases in which careful study revealed no abnormality in these areas. Schröder² described demyelination not in plaques but along the course of nerve bundles, although it was of varying intensity even in the length of a given bundle. Levaditi, Hornus and Schoen^{6b} described demyelination in

plaques, with thickening of the vessel walls and perivascular collections of monocytes. Gärtner^{6f} found demyelination in the temporal half of the optic nerves. Davison⁵ considered the lesions in the chiasm and optic tracts similar to those in the hemispheres except for the absence of "gemästated glia cells." Scherer expressed the belief that the most constant finding is papillomacular degeneration, sometimes complicated by plaque-like foci.

Changes were present in the spinal cord in the majority of cases, although absent in a few carefully examined cases and not mentioned in others, in which the cord was evidently not examined. Most authors have described demyelination of the posterior columns, accompanied by some loss of axis-cylinders and the presence of fat-containing cells. This usually occurred in a roughly triangular area, affecting the more dorsal portions of the posterior columns at all levels of the cord except the lower lumbar and sacral. Frequently similar, though less severe, degeneration of the pyramidal tracts has been described. Scherer,⁷ however, pointed out that the lesions of the cord are of two types—"funicular foci" of demyelination with numerous gutter cells and status spongiosus with minimal tendency toward glial scarring, and secondary degeneration of tracts elsewhere affected by these foci. The plaques were most common in the thoracic region and may occur, in posterior, lateral or anterior columns. Secondary degeneration may be present in cervical and lumbar regions. The plaques did not necessarily include the entire cross section of a given tract, nor were they always limited to single tracts.

Few studies have been made of the nerve roots and peripheral nerves. Gärtner^{6f} described swelling of myelin sheaths and many lipophages in the sciatic nerves. Schob^{6c} mentioned degeneration of posterior roots. Levaditi, Hornus and Schoen^{6h} found satellitosis and neuronophagia, but no demyelination, in the spinal ganglions. Scherer⁷ carefully studied the roots and peripheral nerves in all his cases and only rarely found changes—noninflammatory fascicular degeneration of posterior roots and sciatic nerve. He also studied the roots and nerves in many other monkeys and never found these changes in the absence of the disease under discussion.

The cause, the nomenclature and the extent to which the disease is related to various human diseases have occasioned much difference of opinion. Rothmann¹ suggested that it might be a form of tabes, but later workers have denied the possibility of syphilis. Schröder² considered it not identical with any human disease and merely conjectured that it was caused by some injurious substance acting on the myelin. Steiner,⁴ as previously noted, considered it identical with multiple sclerosis, while Perdrau^{6a} thought it the same as Schilder's disease (progressive subcortical encephalopathy). Schob compared it to both

of these but decided that it was identical with neither and merely described it as a spontaneous disease of unknown origin. Davison⁵ agreed with this point of view, though suggesting the possibility of a disorder of lipid metabolism.

Funicular myelosis, or subacute combined degeneration (dorsolateral spinal degeneration), is the human disorder with which this primate disease has been identified by Scherer,⁸ Von Bogaert⁶ⁿ and Hamerton.⁹ They pointed out the similarity of the spinal lesions and the fact that cerebral lesions have been frequently described in man, although much less often than in the monkey. Studies of the blood of monkeys have been made infrequently and when made did not show the findings of pernicious anemia.

In only 3 instances⁶ⁿ were stomach contents examined for hydrochloric acid. Achlorhydria was found in all 3. Nutritional disorders have frequently been noted. Hamerton⁶ⁿ apparently achieved cure in 1 case in which the clinical symptoms were typical, by injections of a purified liver solution (campolon[®]). Scherer⁷ produced marked clinical improvement in 1 case by improving the diet. Although the authors admitted that these isolated cases may represent spontaneous remissions, they expressed the belief that the evidence strongly suggests nutritional deficiency as the cause.

The possibility of an infectious basis has been stressed by Gärtner^{6e, f} and by Levaditi and his colleagues,^{6g, h} who mentioned the epidemiologic evidence. A group of orang-utans was brought to Europe from Sumatra. Many of these died, including the one observed by Schob^{6c} and others with similar symptoms at Halle, Germany. Later a similar disease developed in a *Cynopithecus maurus* in a cage near the sick orang-utans, and still later 2 baboons housed nearby showed the same symptoms. Levaditi, Hornus and Schoen^{6g} described a group of 120 monkeys brought from Simla, capital of Delhi province, India, to Milan, Italy, in 1932, 12 of which became sick with presumably the same condition. Four of Scherer's animals were members of a group of 10, all of which got sick on the same transport.

Levaditi, Hornus and Schoen⁶ⁱ attempted to transmit the disease experimentally. They prepared an emulsion of the brain of a monkey with typical findings and injected it intracerebrally into a chimpanzee, a *Cynocephalus babuin*, a *Cercopithecus callithrix*, a *Macacus cynomolgus*, and 2 rhesus monkeys. No positive changes occurred in any of these except 1 rhesus monkey which four months after the inoculation became weak and hyperexcitable to touch and died one month later. At autopsy hemorrhagic foci were found in the basal ganglions

8. Footnotes 6d, 6m and 7.

9. Footnotes 6j, 6k, 6l, 6n and 6o.

and the choroid plexus and at the base of the brain. The lesions were of two types—acute lesions marked by thrombosed vessels, polymorphonuclear leukocytes infiltrating the vessel walls, some necrosis and numerous lipophages, and chronic lesions with demyelination and increased oligodendroglia and cells containing fat granules. They considered this a successful transmission experiment and suggested that the other experimental animals failed to contract the disease because they were not of the same species as the original source of the infective material. However, since both the clinical and the pathologic evidences of this disorder were quite different from those of the spontaneous disease, we do not believe that this can be considered evidence of transmissibility. Gärtner^{10, 11} also attempted to transmit the disease. He injected spinal fluid from a sick animal intramuscularly into a rhesus monkey, in which in a few weeks typical symptoms developed, and at autopsy areas of destruction of axis-cylinders and increase of astrocytes, with no inflammatory changes, were demonstrated. He mentioned making a second successful passage from the experimental animal to another. Unfortunately, Gärtner's work is reported in two brief papers, lacking in detail, and we can find no evidence of its ever having been repeated or confirmed. We do not believe there is any convincing evidence that the disease can be transmitted. The evidence of at least a pseudo epizootic occurrence might have an infectious origin or might be related to dietary conditions.

In summary, a wide variety of apes and monkeys have been affected by a demyelinating condition affecting brain, optic nerve and tracts, and spinal cord, of subacute to chronic course. In spite of diversity of pathologic description, all the reported animals appear to be suffering from the same disease entity. The distribution of cases suggests an epizootic nature of the disease, but no infectious or transmissible agent has ever been satisfactorily demonstrated. Dietary or other environmental factors are suggested as etiologic but have not been proved to be the cause.

SWAY-BACK

A nervous disease of newborn and suckling lambs has been noted in various parts of the world since, perhaps, as long as one hundred years ago.¹⁰ It has been known by various popular names—sway-back, swing-back, *warfa*, *renguera*, Gin Gin rickets—but was not studied scientifically until recent years, when it became a serious economic problem.

In 1917 Gaiger¹¹ published a study of *renguera* occurring in the Sierra region of Peru. The name of the disease was said to be derived

10. Innes, J. R. M.: Personal communication to the authors.

11. Gaiger, S. H.: J. Comp. Path. & Therap. **30**:185, 1917.

from words meaning "broken back." He stated that young lambs aged from 6 weeks to several months were affected, showing weakness and incoordination of the hindlimbs, sometimes tremors of the head. Death usually ensued. He found no constant autopsy results except some increase in the color of the meninges, but apparently his pathologic examinations were casual and incomplete. There was evidence suggesting that a micrococcus might be the cause, but this was not confirmed by subsequent work.

In 1920 Magnusson¹² described a disease occurring in nursing lambs in Sweden with similar symptoms. He mentioned a few cases which had been noted elsewhere and expressed the belief that the condition might be the same as *renquera*. He, too, failed to find significant pathologic changes. Transmission experiments were unsuccessful. Innes¹⁰ suggested that this may not be the same disease, since no subsequent cases have been observed in Sweden.

In 1932 two significant papers appeared. Bennetts¹³ described what he called enzootic ataxia, which had been noted in certain parts of Australia for twenty years. It occurred in lambs from 6 weeks to 4 months of age and was characterized by ataxia and malnutrition, leading to death. Farmers had noted the disease only in lambs born of ewes which had pastured in certain affected areas during pregnancy. The disease could be prevented by sending the ewes to a different locality for part of their pregnancy. Bennetts found no lesions in the brain but observed consistent changes in the spinal cord following Marchi stains. He stated that there was "definite degeneration of certain nerve fibres in the thoracic and lumbar spinal cord" and that "scattered fibres in the femoral and sciatic nerve and its branches were also degenerated." In some cases the disease ran a prolonged clinical course, and in these cases he observed definite "sclerosis" of the cord.

In the same year Stewart¹⁴ wrote a paper on "swing-back," which he stated occurred all over Britain but especially in southern Scotland and northern England. He also described the incoordination of the hindlimbs, which he said was made worse by excitement. The mildly affected lambs could walk a short distance but when driven farther would fall exhausted. If left alone, they would soon be able to get up and advance another short distance. If the condition appeared in one of twins, the other twin was certain to show the disease also. Stewart distinguished two types of the disorder. In the congenital form symptoms appeared shortly after birth; the lambs were unable to rise or suckle and soon died. The chronic type, which more closely

12. Magnusson, H.: Deutsche tierärztl. Wchnschr. 28:297, 1920.

13. Bennetts, H. W.: Australian Vet. J. 8:137 and 183, 1932.

14. Stewart, W. L.: Vet. J. 88:133, 1932.

resembled the condition described by Gaiger and by Bennetts, appeared when the lambs were somewhat older—10 to 14 days—and had a longer course. He described tracts of degenerated fibers in the medulla and cord, especially the thoracic part of the cord. He believed that the affected portions were homologous to the direct and ventrocerebellar tracts and the column of Goll (*fasciculus gracilis*). He also mentioned an "abnormal condition of the cells of Clarke's column" at the thoracic level.

Between 1937 and 1944 a number of important contributions were made by Innes and co-workers¹⁵ in England, who firmly and for the first time established the pathologic foundation, and by Bennetts and his colleagues¹⁶ in Australia. The disease as observed in England appeared more acute than the Peruvian and Australian forms, occurring in newborn or very young lambs. Innes and Shearer^{15c} noted lesions of the brain rather consistently in their cases. In the most severe form there was symmetric gelatinous degeneration of the white matter of the hemispheres, frequently with cavitation. In the earlier stages a type of demyelination occurred, which was compared to Schilder's disease. Histologically, the demyelination occurred rapidly, and axis-cylinders were destroyed as quickly as myelin. There was not much accumulation of stainable lipoids, nor any marked perivascular cellular infiltration. No inclusion bodies, neuronophagia or evidences of inflammation were found. The nerve cells were not damaged except for chromatolysis in the red nucleus, which these authors considered related to the lesions of the spinal cord. Where cavities occurred, the walls were composed of glia fibers, and fibrous astrocytes were noted. There was not much evidence of microglial participation. Degeneration of the motor tracts of the cord was noted. Innes concluded that the disease was of antenatal origin but that it must begin late in gestation. His studies showed the disease to be true degeneration of myelin and not aplasia. The tendency to liquefy was due to the characteristic reaction of infantile tissue. Culture and transmission studies repeatedly failed to give any evidence of an infectious etiologic agent. In healthy lambs which had been suckled by the mothers of sway-back lambs the disease did not develop.

15. (a) Innes, J. R. M.: *J. Neurol. & Psychiat.* **2**:323, 1939; (b) *Proc. Roy. Soc. Med.* **33**:169, 1940. (c) Innes, J. R. M., and Shearer, G. D.: *J. Comp. Path. & Therap.* **53**:1, 1940. (d) Shearer, G. D.; Innes, J. R. M., and McDougall, E. I.: *Vet. J.* **96**:309, 1940. (e) Innes, J. R. M.: *Vet. Rec.* **55**:369, 1947. (f) Shearer, G. D., and McDougall, E. I.: *J. Agric. Sc.* **34**:207, 1944.

16. (a) Bennetts, H. W., and Chapman, F. E.: *Australia Vet. J.* **13**:138, 1937. (b) Bennetts, H. W., and Beck, A. B.: Bulletin 147, Council of Scientific and Industrial Research of Australia, 1942, p. 1.

The first clue to the etiologic explanation was announced by Bennetts and Chapman^{10a} in 1937. Several investigators had suspected that lead might cause the condition because most of the areas where it occurred had relatively high concentrations of lead in the soil, although the diseased animals showed none of the usual findings of plumbism. In the hope that the animals might be "de-leaded" they were treated with ammonium chloride, and although at first the results seemed promising they were most inconsistent. Further investigation showed that the commercial preparation of ammonium chloride being used contained various impurities, including copper. Further studies revealed that chemically pure ammonium chloride was of no prophylactic or therapeutic value, but that if ewes were given extra copper, the disease was prevented in their lambs. In one experiment 12 ewes received a daily drench of copper sulfate. In none of their lambs did the ataxia develop. A control group of ewes was kept under similar conditions without the added copper, and the lambs showed a 100 per cent incidence of ataxia. These investigators also found that if the sick lambs were treated adequately with copper this disease was apparently cured. They noted macrocytic anemia in the pregnant ewes in the affected area and an abnormally low copper content in their liver, blood and milk. Similarly, the liver of the diseased lambs was low in copper. On the basis of their findings, Bennetts and Chapman suggested that there was a similarity between this disease and pernicious anemia, a concept which in view of later work does not seem to be valid.

Bennetts and Beck^{10b} presented a longer and more detailed study in 1942. They noted that the disease was confined to the progeny of ewes which had been in the affected area at least six months and that the incidence of the disease increased the longer and more heavily a given region was stocked. Whereas in their previous Australian studies they observed that all the cases concerned lambs 6 weeks old or more and that none had lesions of the brain, they now reported several instances of a much more acute form of the disease. Lesions of the brain were found, with fluctuant softening and cavitation of the white matter. Four of these brains were examined by Innes, who agreed that the cases were similar to the English cases. In most animals degeneration of myelin occurred from the middorsal to the midlumbar regions of the spinal cord, particularly in areas which corresponded to the direct cerebellar tracts and Lissauer's tract (*fasciculus dorsolateralis*), but not definitely systematized. Damage of the gray matter was not found. Some scarring was observed in chronic cases.

Bennetts and Beck reported several further experiments indicating the etiologic role of copper deficiency. They showed that in the affected areas there was an abnormally low copper content of soil and herbage; that the ewes and affected lambs had levels of copper below the average,

and that the disorder might be prevented by supplying extra copper to the pregnant and lactating ewes and sometimes cured by giving it to the lambs. They also noted that the ewes with copper deficiency showed stringy wool and that the wool improved when extra copper was supplied. Anemia was frequent but not constant in the diseased animals.

Innes and co-workers¹⁵ in several experiments confirmed the belief that copper was prophylactic and that in the blood and liver of the affected animals copper levels were low. In contrast to the findings in Australia, stringy wool was never noted in England. Further, there was no evidence that the copper content of the soil or herbage of those areas in England where the disease was most prevalent was unusually low. It was noted that ewes of Derbyshire, where the disease was common, if moved to Cambridge, where it did not occur, showed increase of copper and bore normal lambs, although repeated examination of the herbage in the two areas showed similar copper contents. Innes and his co-workers felt that the role of lead could not be entirely ruled out, possibly as affecting the utilization of copper. They also speculated on the significance of the calcium-phosphorus ratio. Innes^{15e} published an interesting review of the studies to date in 1943.

McDonald,¹⁷ in 1942, summarized the findings on this disease in Australia. In general, he supported the findings of the other workers and showed that the condition occurred in South Australia. He made autopsies in 22 cases, in 1 of which he found cavitation in the hemispheres, but in the others he noted changes only in the cord, except for the degenerative changes found in cells of the red nucleus. He described similar changes in the large multipolar cells of the ventral horns of the spinal cord and in the medulla. He noted that although anemia may be present, it is not an essential part of the syndrome, and he explained that sway-back often is complicated with a disease due to cobalt deficiency, which may result in anemia. He suggested that the difference in frequency of lesions of the brain as described by Innes and the Australian investigators is a reflection of a difference in intensity of the disease. He also described 1 case in which sway-back occurred in a 4 year old animal which had been under careful observation all its life. Ataxia developed, and one month later the animal was killed and examined. Typical demyelination and nerve cell changes were found.

Also in 1942 there appeared an extensive review of *renguera*, by Tabusso¹⁸ in Peru. Neuropathologic studies confirmed the belief that

17. McDonald, I. W.: Australian Vet. J. 18:165, 1942.

18. Tabusso, M. E.: Publ. Inst. nac. biol. anim., January 1942, vol. 1, no. 1; Vida Agri, October 1941 (This reference we have been unable to locate in any library, but through the courtesy of Dr. Innes a reprint was made available to us.)

it was identical with sway-back. He mentioned that copper had been shown to have prophylactic value but did not describe experiments and was not convinced of the etiologic importance of copper deficiency. He referred to the successful transmission experiments of Mitchell suggesting a virus as the cause, but this suggestion was not confirmed. Innes^{19c} expressed doubt whether Mitchell was really dealing with *renquera*, and not with loup-ill.

In 1947, in an interesting paper,¹⁹ it was reported that four of the seven investigators in England most active in these studies had subsequently suffered from nervous diseases resembling multiple sclerosis. This, of course, suggested the possibility of an infectious agent which acted only in the presence of a "trace element" deficiency. There has been no other evidence that sway-back was infectious. Transmission experiments have consistently failed to reproduce the disease, and there is no evidence of contagion. The paper in question, although of interest, does not rule out coincidence and adds no real proof of the infectious nature of sway-back.

In summary, sway-back is a disease of young lambs characterized clinically by ataxia and pathologically by demyelination in the spinal cord, chromatolysis of cells of the red nucleus and sometimes of the ventral horns, and, in the more acute cases, symmetric gelatinous softening leading to cavitation of the white matter of the hemispheres. There is convincing evidence that copper deficiency in the pregnant ewes plays an etiologic role and that the disease may be prevented by giving copper supplements to the ewes, but the mechanism by which the copper is effective is not known.

DEMYELINATION IN HORSES

A peculiar noninfectious nervous disease of horses has been observed by veterinarians from time to time since at least 1891.²⁰ Buckley and MacCallum²¹ in 1901 and 1902 described what they called first "acute hemorrhagic encephalitis," later "acute epizootic leucoencephalitis," prevalent among horses in Maryland. They stated that, according to most observers, the disease occurred when horses were fed moldy fodder. Clinically there was some variation in symptoms, which included drowsiness, impairment of vision, paralysis of the pharynx, muscular twitching, unsteadiness of gait and weakness, followed by coma

19. Campbell, A. M. G.; Daniel, P.; Porter, R. M.; Ritchie, R. W.; Smith, H. V., and Innes, J. R. M.: *Brain* **70**:50, 1947.

20. (a) Mayo, N. S., in discussion on Schwarte.^{24d} (b) Mizelle, J. D., and Graham, R.: *Cornell Vet.* **27**:374, 1937.

21. (a) Buckley, S. S., and MacCallum, W. G.: *Am. Vet. Rev.* **25**:99, 1901. (b) MacCallum, W. G., and Buckley, S. S.: *J. Exper. Med.* **6**:65, 1902.

or delirium. The course was rapid, death sometimes occurring within three hours after onset of symptoms, sometimes after as much as one week. The average duration was about three days. Most affected horses died. The few which recovered showed diminished intelligence or were "dummies." Buckley and MacCallum studied the brains of 4 animals which had died during the acute attack and on which autopsies were made; cultures of the brain material were negative. The meninges were slightly congested or normal, and there was no inflammatory exudate. The gray matter was always normal. In the white matter of the brain there were soft, fluctuating areas containing a grayish yellow pulpy mass of necrotic tissue and glairy opalescent fluid, mixed with dark blood. Adjacent to the cavities there were numerous hemorrhages, and microscopically there were loss of nerve tissue with persistence of glia cells and a moderate exudation of leukocytes. Axis-cylinders showed degeneration, swelling and thickening but persisted fairly well into the necrotic areas, where they terminated abruptly. The myelin sheaths were broken up into globules which took myelin stains. Disintegration of glia cells were described and the presence of a few fat granule cells noted. None of the large blood vessels were occluded, but the walls of many small vessels were infiltrated by leukocytes and round cells, and lumens were often occluded by hyaline thrombi. Buckley and MacCallum also made an autopsy of one of the recovered "dummies" and found a grayish translucent ramifying scar in the white matter of the anterior part of the cerebrum, which microscopically was composed of loose fibrous tissue.

In 1902 Butler²² described the same disease and reported an experiment in which 2 healthy colts were fed moldy corn from a farm where 4 horses had died. One colt became ill twenty-six days after the experiment began and died in three hours. At autopsy the white matter of the left cerebral hemisphere was almost entirely broken down and the right hemisphere contained a small area of necrosis.

Dexler,²³ in 1903, published a study of hemorrhagic encephalitis of unknown origin occurring in horses in which there were disseminated areas of softening. It is impossible to determine whether he was discussing the same disease.

Although there was an outbreak of the disease in Illinois in 1914 and sporadic cases occurred in other corn belt states, not much further attention seems to have been paid to it until a serious epizootic occurred in 1934-1935, during which about 5,000 horses and mules died in Illinois and heavy losses occurred in Iowa. In the next few years a

22. Butler, T.: *Am. Vet. Rev.* **26**:748, 1902.

23. Dexler, H: *Monatschr. f. Neurol. u. Psychiat.* **13**:97 and 210, 1903.

number of papers dealing with this condition²⁴ appeared, mostly from Illinois and Iowa, and the following description is based on them.

The clinical syndrome varied in intensity. Graham and co-workers^{24a, b} described three types—lethargic, nervous (or excited) and paralytic—and remarked that the clinical syndrome resembled that of virus encephalitis. Schwarte and co-workers^{24c} also noted the similarity of this disease and virus encephalitis and pointed out that in the absence of autopsy the diagnoses are often confused. They mentioned as possible symptoms depression, incoordination, rolling on the ground, walking in circles and violent motor activity.

The autopsy findings outside the central nervous system were essentially without significance except that Schwarte and co-workers^{24c} mentioned evidence of severe toxic changes in liver, kidneys and bladder. The brain showed softening or liquefaction unilaterally or bilaterally in the white matter. In the areas adjacent to the necrosis, edema and hemorrhages were noted, but no perivascular cuffing or round cell infiltration. Graham described degenerative changes of the nerve cells, and Schwarte mentioned some increase of glial elements. Occasionally microscopic hemorrhages, edema and degenerative changes occurred in the spinal cord.

All observers agreed that the disease seems to be related to the ingestion of moldy corn and that severe outbreaks are most likely to take place when weather conditions have been favorable for the growth of molds on the corn. Although often called "cornstalk disease," it appears that not only the stalks but the ears and the kernels may carry the causative agent. Experiments have been made without success to determine what this is. Butler's²² experiment has been noted. Graham^{24a} placed 8 horses in a stalk field where there had been previous occurrence of the disease. Two of the 8 horses died with more or less typical symptoms, and an autopsy of 1 horse was made, which showed the characteristic findings. Schwarte^{24d} fed 5 horses moldy corn. Typical symptoms developed in all, and all showed the usual postmortem changes, while 3 control animals remained healthy. Biester and co-workers^{24f} experimented with corn showing different types of mold, one predominantly green, the other gray, and with both produced some clinical symptoms and fairly typical autopsy findings. Feeding experiments were made with various molds isolated from corn and corn chaff

24. (a) Graham, R.: *Vet. Med.* **31**:46, 1936. (b) Graham, R., and Harris, B.: *ibid.* **31**:340, 1936. (c) Schwarte, L. H.; Biester, H. E., and Murray, C.: *J. Am. Vet. M. A.* **90**:76, 1937. (d) Schwarte, L. H.: *ibid.* **92**:152, 1938. (e) Biester, H. E., and Schwarte, L. H.: *North Am. Vet.* **20**:17, 1949. (f) Biester, H. E.; Schwarte, L. H., and Reddy, C. H.: *Vet. Med.* **35**:636, 1940. (g) Biester, H. E., and Schwarte, L. H.: *ibid.* **39**:303, 1944. Mizelle and Graham.^{20b}

by Mizelle and Graham^{20b} without positive results. All experimenters have agreed that it is impossible to isolate any virus or to transmit the disease by inoculation of brain. Graham and Harris isolated *Pasteurella* from 2 cases of the disease, naturally occurring, but there has been no further indication that these organisms play a causative role.

It has been noted that cattle and other animals are not made ill by the moldy corn which affects horses, but that in cattle a different disease may develop from consumption of other types of abnormal corn which are not harmful to horses.²⁵

This disease has received little attention in neurologic or pathologic literature. Detailed neuropathologic and topographic analysis of lesions have not been carried out. Little has been added to the original pathologic description of MacCallum of 1902, in spite of the great advances in neuropathologic methods and technics since that time and the apparent great abundance of cases. Of present day neuropathologists, Hurst²⁶ alone appears to have had access to material. In general he confirmed the findings of MacCallum and Buckley.^{21b} He noted that in the most severe portions of the lesions all nuclear staining was lost. Elsewhere early microglial reaction, with swelling and proliferation of adventitial cells and capillary endothelium, was observed. The central areas exhibited almost complete demyelination, with no axis-cylinders remaining. In the outer zones the myelin sheaths were varicose. Compared with lesions which he produced experimentally in monkeys by administering potassium cyanide, the lesions of the horses showed more complete central softening, a less abrupt transition from softening to a normal condition, and involvement of arcuate fibers.

Fundamentally the disease appears to be of toxic character, but the means by which the ingestion of the toxin is translated into catastrophic destruction of white matter is not clear. The pathologic changes, including hemorrhages, thrombi and presence of polymorphonuclear leukocytes, suggest a vascular mechanism. But the described changes do not resemble the picture associated with massive thrombosis, nor are the clinical and the experimental course of the disease consistent with cerebral thrombosis as commonly understood. The peculiar localizing of lesions to the white matter compels one to include this condition in the category of spontaneous demyelinating diseases.

DEMYELINATION IN MOOSE

An obscure condition affecting moose has received sporadic attention in various fields of research. The relevant literature has been

25. Schwarte, L. H.; Eveleth, D. F., and Biester, H. E.: *Vet. Med.* **34**:648 1939.

26. Hurst, E. W.: *Australian J. Exper. Biol. & M. Sc.* **18**:201, 1940.

reviewed and the neuropathologic findings described by King.²⁷ The affected animals, observed in Maine and Minnesota, exhibited weakness, staggering, incoordination and drowsiness. There has been a trend toward seasonal incidence, with the majority of cases being observed between February and May, but sick animals have been found during other months as well.

It is probable that not all the observed sick animals were suffering from a single disease entity. King studied material from 3 animals of Maine, 2 of which showed similar lesions, while the third revealed none. Brain tissues from 5 sick animals of Minnesota were also studied, with positive results in 3 cases. Thus, of 8 cases examined, only 5 were positive for demyelination; in 4 of these characteristic changes were revealed, while the fifth was atypical. Fenstermacher and Jellison²⁸ are insistent that "the losses of moose that occur in Minnesota are not the result of a single pathogen."

It is not possible, therefore, to maintain that all the various studies of sick moose have dealt with the same disease entity. Experimental work carried out in cases in which demyelination was demonstrated has failed to exhibit any transmissible agent or to provide evidence of infection.

Pathologic examination showed damage of the white matter. Abundant neutral fat was observed in poorly defined foci. In some areas the fat was diffusely scattered through the involved tissues, while in others it had a predominantly perivascular distribution. Myelin stains showed zones of poorly circumscribed loss, with relative preservation of axis-cylinders. Fibrous gliosis was found. In the white matter of the cerebrum rather sharply defined perivascular scars were exhibited by appropriate glial stains. In the cerebellum and the medulla more diffuse gliosis was found, quite disproportionate to the amount of myelin lost. In the 4 typical cases cellular infiltrations were noted, virtually limited to the white matter and generally perivascular in distribution. The fifth case, with inflammatory involvement of the cortex, appeared atypical.

Examination showed evidence of a process of a relatively mild type, with destruction of myelin and fibrous gliosis, not sharply circumscribed, and accompanied in more acute phases by variable degrees of cellular infiltration. The cause is not known. All the available evidence points away from an infection and suggests the possibility of dietary deficiency or possibly the action of some toxic substance. This condition appears to be a disease entity responsible for the death of an unknown per-

27. King, L. S.: *Am. J. Path.* **15**:445, 1939.

28. Fenstermacher, R., and Jellison, W. L.: *Diseases Affecting Moose*, Bulletin, University of Minnesota Agricultural Experiment Station, 1933, p. 294.

centage of native moose. To our knowledge the disease has not been found in moose in captivity.

ENCEPHALOMYELITIS IN THE DOG

The problem of demyelination is more complex in the dog than in the animal species discussed hitherto. The demyelinations observed in monkeys, sheep, horses and moose represent, in all probability, single discrete disease entities. In the dog there is a variety of poorly defined diseases involving the nervous system. Demyelination is a feature of some but not of all of these involvements.

The confusion has been pointed out by Innes,¹⁰ who distinguished six groups of nervous disorders in dogs, separable on pathologic, although not necessarily on purely clinical, grounds. These groups include (a) brains and cords without lesions, suggesting true canine hysteria, (b) inflammatory changes with demyelination, (c) vascular disturbances with blood vessel proliferation in the gray matter, (d) encephalitis with perivascular lymphocytic infiltration, (e) chronic lymphocytic meningitis and (f) true distemper virus infection. Unfortunately, in the literature most cases of cerebral involvement have been uncritically assumed to be instances of distemper virus infection and have been described as such.

Distemper is a specific disease entity, caused by a filterable virus. It is a highly infectious disease of dogs but may also, according to some authors, attack fox, wolf, hyena and monkey,²⁹ ferret, stoat and weasel.³⁰ Bacteria are recognized as secondary invaders. The encephalitozoon described by Kantorowicz and Lewy³¹ is not etiologic. Clinically the disease shows a varied pattern of symptoms. In classic cases there is an initial rise of the temperature seven to eight days after exposure to infection, with recession and then a secondary rise, giving a diphasic fever. There is usually associated coryza, as well as frequent catarrhal symptoms of eyes and nose. Bronchitis is common, and bronchopneumonia may be present. Cutaneous lesions may occur varying from vesicular to pustular. Diarrhea and vomiting, or "gastro-intestinal catarrh," may be prominent. Inclusion bodies are of diagnostic importance, but their consideration lies outside the scope of this paper. One may refer to de Monbreun,³⁰ Green and Evans³² and Watson and Plummer.³³ In some outbreaks nervous manifestations may appear.

29. Marinesco, G.; Dragenesco, S., and Stroesco, G.: *Ann. Inst. Pasteur* **51**: 215, 1933.

30. de Monbreun, W. A.: *Am. J. Path.* **13**:187, 1937.

31. Kantorowicz, R., and Lewy, F. H.: *Arch. f. wissenschaft. u. prakt. Tierh.* **49**: 137, 1923.

32. Green, R. G., and Evans, C. A.: *Am. J. Hyg.* **29**:73, 1939.

33. Watson, E. A., and Plummer, P. J. G.: *Am. J. Vet. Research* **3**:350, 1942.

According to Pugh, these nervous symptoms may occur weeks or months after the initial or primary symptoms, or may be seen without any of the primary systemic disturbance. According to de Monbreun, the nervous symptoms, if they occur, usually develop after the second week of the disease.

The clinical picture of distemper is so variable that veterinarians sometimes divide the disease into various types, catarrhal, intestinal, cutaneous or tegumentary, and nervous, depending on which system is most prominently affected.

All observers agree that the disease is prevalent throughout the world and that subclinical or atypical infections are frequent. So great is the variability that Dunkin and Laidlaw³⁴ declared it may be impossible to diagnose distemper (clinically) with certainty. To prove distemper it is necessary to rely not on clinical judgment but on biologic studies in which virus-containing materials are inoculated and protection tests made. Further difficulty is offered in the choice of a suitable test species. Dogs, unless very young, are not suitable, because they may have been immunized by previous subclinical infection. Ferrets, as demonstrated by Dunkin and Laidlaw, are the animals of choice. Thus, extreme practical difficulties attend the positive identification of this disease. It is probable that even skilled veterinarians are not entirely reliable in their clinical diagnosis of distemper. Certainly, in cases of the so-called atypical or abortive type the diagnosis cannot be accepted without rigorous proof. As Innes^{15b} said, "The terms 'distemper' and 'post-distemper encephalitis' have been loosely applied and with no more proof than that the dog may, some time previously, have suffered from a febrile illness." We may say that the diagnosis of canine distemper bears the same relation to canine disease that the loose diagnosis of "flu" does to human febrile illnesses.

The most comprehensive pathologic description of the brain involved in alleged distemper was written by Cerletti³⁵ in 1912. Little of significance has been added by subsequent writers. Cerletti personally observed 32 dogs both clinically and pathologically; 28 of them had essentially "typical distemper"; in 10 it was clinically purely "catarrhal," while in 18 it assumed clinically the "nervous" form. The remaining 4 dogs showed nervous symptoms several months after their alleged distemper, and Cerletti admitted an assumption in relating these to the other groups. For our purposes these 4 dogs are inadmissible. Of the 10 dogs with the catarrhal form but without clinical nervous symptoms, 6 showed in the nervous system localized areas of intense plasma

34. Dunkin, G. W., and Laidlaw, P. P.: *J. Comp. Path. & Therap.* **39**:201 and 213, 1926.

35. Cerletti, U.: *Arch. f. d. ges. Neurol. u. Psychiat.* **9**:520, 1912.

cell and lymphocytic accumulation around pial vessels and infiltrating into nerve tissue, together with glial proliferation. These findings could not be qualitatively distinguished from those in the "nervous form." In the latter the inflammatory changes in the nervous system were more intense and widespread. Cerletti somewhat artificially distinguished three types of change, designated as focal (perivascular) infiltrations, "productive" foci characterized by proliferation of vascular adventitial and endothelial cells, and foci or nodules of glial proliferation. These occurred singly or in combination.

Today, with vastly increased knowledge of the pathologic aspects of virus encephalitides, these findings are entirely consistent with virus causation. With additions they have been confirmed by Hurst and co-workers,³⁶ who studied both the neuropathologic and the immunologic aspects.

It is of great interest that with regard to the inflamed areas Cerletti described and illustrated foci and areas of fat granule cells (*Körnchenzellen*). The legend of one of his illustrations, figure 10, shows the misprint *Körbchenzellen*. Beyond all reasonable doubt such zones were areas of demyelination such as were described later by Perdrau and Pugh³⁷ and others.

Cerletti's work, as mentioned in a foregoing paragraph, was in essence confirmed by Gallego³⁸ and others, who, however, did not evince any interest in the status of the myelin. Roman and Lapp³⁹ confirmed the fact that inflammatory lesions occur in the brain in the absence of clinical neurologic signs. None of these writers were critical of the accuracy of the diagnosis of distemper.

Perdrau and Pugh,³⁷ in 1930, reporting for the first time demyelination as observed in the dog, opened up a new era in canine neuropathology. They studied 14 animals, all suffering clinically from nervous disorder and showing pathologically disseminated encephalomyelitis, that is, scattered zones of inflammatory reaction involving the brain stem and spinal cord. Of these 14 animals, 7 had a history pointing to a previous attack of distemper. Of the total series, 3 from the distemper group and 1 of the remaining group showed demyelination as part of the disease process.

The authors pointed out that distemper has long been associated with encephalitis, known as "the nervous form of distemper," supposedly due to spreading of the virus to the brain. From the clinical standpoint they did not believe that in their cases a causal relation

36. Hurst, E. W.; Cooke, B. T., and Melvin, P.: *Australian J. Exper. Biol. & M. Sc.* **21**:115, 1943.

37. Perdrau, J. R., and Pugh, L. P.: *J. Path. & Bact.* **33**:79, 1930.

38. Gallego, A.: *Ztschr. f. Infektionskr.* **34**:38, 1928.

39. Roman, B., and Lapp, C. M.: *Bull. Buffalo Gen. Hosp.* **3**:40, 1925.

could be established between the virus disease and the demyelination. For example, one of their dogs, a puppy 8 months old, had always been well until it came in contact with an animal suffering from distemper. The puppy speedily became ill, exhibited nervous symptoms and was killed on the fourth day of illness. The brain showed chronic patches of demyelination, clearly older than the duration of the illness.

Perdrau and Pugh expressed the belief that "the encephalomyelitis commonly referred to as 'the nervous form of distemper' is not the result of the action of the specific virus of distemper on the central nervous system, but that the virus of distemper plays in this disease of the dog a similar role to that which an acute infection of varying etiology plays in the causation of certain demyelinating diseases of man."

That the animals described by Perdrau and Pugh may not have been suffering from distemper received emphasis from Laidlaw,⁴⁰ who was unable to reproduce the disease in ferrets with brain tissue from some of the animals of Pugh.

Additional evidence concerning demyelination was speedily forthcoming. Marinesco and associates,²⁹ as well as Peters and Yamagiwa,⁴¹ also described demyelinating changes of the brain in addition to inflammatory involvement. The loss of myelin occurred predominantly in the cerebellar peduncles, the medulla and the pons. Both of these authors claimed to be describing distemper encephalitis. But Marinesco and co-workers did not state the source of their material, and Peters and Yamagiwa did not exercise a sufficiently critical attitude in their analysis of their cases.

On the other hand, Scherer and Collet⁴² emphasized the importance of skepticism in regard to distemper as a cause. They described 3 cases in which damage of the brain was observed, in dogs, all quite different. Only their second case was similar to those described in the foregoing paragraph, with disseminated foci of inflammatory change and demyelination involving pons, medulla, thalamus, hypothalamus, internal capsule and parietal white matter. The dog was sick for only three weeks, but the visceral organs showed no change. They expressed the belief that distemper was not the cause. Their third case, in which the clinical symptoms lasted for eighteen months, beginning one month after an attack of alleged distemper, is apparently unique in the literature. There was strictly perivascular inflammation,

40. Laidlaw, cited by de Monbreun.³⁰

41. Peters, G., and Yamagiwa, S.: *Arch. f. wissenschaft. u. prakt. Tierh.* **70**:138, 1936.

42. Scherer, H. J., and Collet, L.: *J. belge de neurol. et de psychiat.* **39**:132, 1939.

with no infiltrations of tissue, but in addition hydrocephalus was present, with glial sclerosis involving the white matter of both hemispheres. This case they considered to be one of a peculiar post-distemper complication. But they emphasized that a histopathologic diagnosis of distemper cannot be made.

The first report of demyelination occurring in the dog to be published in this country was that of King.⁴³ The animal showed paralysis, spasticity, contractures and marked sensory loss. It had been ill for about three months. The lesions were those of disseminated encephalomyelitis, with widespread areas of severe inflammatory infiltrations in the parenchyma, involving all levels from spinal cord to cerebral cortex and optic tracts, but most severe in spinal cord, medulla, pons and cerebellum. In areas of severe myelitis, there was vigorous proliferation of reticulin fibers in addition to the destruction of white matter. In some foci of the cord, severe destruction of the gray matter was present, but ganglion cells were strikingly preserved. In the cerebellum and the medulla the lesions were discrete, generally with severe inflammation in the parenchyma. Different types of loss of myelin were noted, a feature which will be treated in the section with the heading "Comment."

King was unable to correlate the condition with distemper and described it merely as disseminated encephalomyelitis with demyelination.

Further data appeared in the literature which cast doubt on the association of demyelination and distemper. Findlay⁴⁴ quoted from an unavailable thesis of Verlinde to the effect that demyelinating lesions have been found in dogs known to be immune to distemper, but the work in question cannot be consulted. Innes^{15b} referred briefly to 50 dogs that he studied. Their number was later considerably augmented, but all the anatomic preparations sent to Scherer and mentioned in his book, were lost during the war.¹⁰ In his published note he stated conservatively that "more than one entity may occur in the dog which may have been regarded in the past as the nervous form of distemper . . . and in which the association of the distemper virus is problematical."

Scherer,⁷ in his recently published book, stated that he did not believe in the direct relationship of distemper and the demyelinating lesions. He considered these lesions to be strictly comparable to acute multiple sclerosis in man, and he contended that this acute multiple sclerosis of dogs has nothing to do with distemper.

Certain experimental studies are of considerable importance. Posrednik⁴⁵ injected supposed distemper virus into 9 dogs and found brain changes indicative of meningitis and meningoencephalitis. But

43. King, L. S.: *Arch. Path.* **28**:151, 1939.

44. Findlay, G. M.: *Proc. Roy. Soc. Med.* **33**:161, 1939.

45. Posrednik, F. I.: *Ztschr. f. Infektionskr.* **38**:136, 1931.

no true demyelination occurred. In contrast, 3 dogs which had died of the natural disease showed severe inflammatory infiltrations with destruction of myelin of the type described by other workers. Posrednik emphasized that in severely inflamed zones there was always disappearance of myelin. Koch's postulates thus were not fulfilled. The severe brain damage and associated demyelination, or loss of myelin in any form, evidently require more than the mere presence of an infectious agent.

De Monbreun³⁰ studied the histopathologic aspects of natural and experimental distemper, paying special attention to inclusion bodies. With a single strain of virus he was able to produce the catarrhal form in some passages, the nervous form in others. In one animal, a puppy dying seventy-two days after inoculation, destructive demyelinating lesions were produced in the medulla and the pons. The photomicrographs are entirely convincing.

De Monbreun's work has been critized by Green and Evans,³² who showed that the virus used in most of the transmission experiments was derived from an animal ill not with canine distemper but with fox encephalitis. This criticism is valid and indicates a difficulty facing research workers, namely, how to determine whether a given sick animal, even one whose brain contains an infectious transmissible agent, is actually suffering from distemper and not from some other disease. The utmost care, with cross protection and immunity tests, and additional use of ferrets, is necessary for accurate work.

For our purpose, however, the point at issue is whether demyelination can be produced by virus action. The problem whether the alleged causative agent is distemper, fox encephalitis or an unnamed virus is less important than that of demonstrating virus causation of some demyelinating conditions in some hosts. De Monbreun's case is not by itself conclusive. Other or accessory etiologic factors have not been excluded. But that a demyelinating lesion was experimentally reproduced with infectious material is an important fact.

A significant contribution is that of Hurst, Cooke and Melvin.³⁶ Hurst studied 9 dogs succumbing in two epidemics of distemper. Seven of the animals with nervous symptoms had suffered from the systemic disorder two or three weeks, 1 six weeks, previously. For 1 dog no history of previous distemper was elicited. The nervous illness lasted three to seven days and terminated fatally. The lesions observed in these brains were similar to those described by other workers, particularly Cerletti,³⁵ Perdrau and Pugh³⁷ and Marinesco and associates,²⁹ with parenchymatous and perivascular inflammatory changes and foci of demyelination. Using material derived from his demyelinated animals Hurst carried out extensive transmission experiments and protection tests, utilizing ferrets as well as dogs. For control

virus he used a strain of distemper virus obtained from the Commonwealth Serum Laboratories in Australia. He and his co-workers were able to show that the brains exhibiting demyelination and encephalitis contained distemper virus. Cross immunity tests were in general satisfactory, although results with a few dogs were not as satisfactory as might be desired. Hurst raised the question whether he was dealing with two closely related viruses. His conclusions are that the cause of "nervous distemper" is "the distemper virus itself, and that the demyelinating lesions produced by it represent damage to the white matter short of complete necrosis."

These positive results he obtained by employing biologic as well as histopathologic methods. Unless the two separated disciplines are employed together, the conclusions reached with either one alone are open to some question. The work of Hurst and co-workers does not solve the problem of the encephalopathy of dogs. His results indicate that at least in some dogs demyelination is caused by virus action, possibly distemper virus, although other viruses have not been definitely excluded.

The work of Hurst has been criticized by Innes,¹⁰ who indicated that in all probability distemper virus was not being employed. According to the latter,¹⁰ recent work by Macintyre, Trevan, and Montgomerie showed that several different infectious agents, with different properties, can be isolated from dogs ill with alleged "distemper." In his forthcoming paper, Innes discusses critically the problem of diagnosis of distemper and the relation of this to other virus diseases.

From our standpoint certain facts stand out. A wide variety of disorders of the nervous system occur in dogs. In the past there has been an unwarranted assumption that such disturbances may be designated as nervous distemper. This assumption must be actively combated. Nevertheless, many febrile infectious illnesses, with or without nervous symptoms, include meningoencephalitis or encephalomyelitis. In some of the dogs demyelination occurs, but demyelination is also seen in other dogs with encephalomyelitis that is unquestionably not distemper.

The common thread here is the presence of inflammation. Most of the foci of demyelination are areas of severe inflammatory change, as emphasized by Posrednik.⁴⁵ There is no inconsistency in holding that the inflammation produced by virus and nonvirus agents may show similar results.

A case in point is described by von Móczy,⁴⁶ who discussed the encephalomyelitis of dogs following rabies vaccination. Such animals

46. von Móczy, J.: Arch. f. wissenschaft. u. prakt. Tierh. 72:15, 1938.

showed widespread severe inflammatory changes. One of his illustrations shows perivascular demyelination. Yet inoculation studies gave entirely negative results, and no transmissible agent could be demonstrated. Here one has demyelination in an inflammatory lesion that is apparently noninfectious but may well be on an allergy or sensitivity basis.

We must conclude with Hurst, Cooke and Melvin³⁶ that demyelination is not specific but is merely an indication of severe damage of tissue. This damage, reflected in inflammatory changes, may lead to loss of myelin. Destruction of myelin therefore is an index of damage to white matter, which unquestionably may have virus localization as one cause but which may have other causes as well. In the dog there is no positive evidence pointing to the nature of these other causes.

ENCEPHALOMYELITIS IN GOATS

The brains of goats suffering from an unusual infectious disease were studied by King.⁴⁷ Over a period of three months a series of 9 goats coming from a single farm in New Jersey all succumbed to an infection. The clinical symptoms lasted only two to three days, and were not distinctive. Specific neurologic signs were rare. The outbreak was studied by TenBroeck and Seastone,⁴⁸ who in 7 of the 9 cases recovered pure cultures of an organism belonging to the genus *Listerella*. Examination of brain tissues showed severe disseminated encephalomyelitis with lesions of predominantly granulomatous character restricted almost exclusively to the brain stem, especially the medulla and the spinal cord. In addition to severe focal parenchymatous nodules, marked perivascular cuffing was noted, and some areas of more diffuse infiltration of tissue. The bacteria could be demonstrated by Gram-Weigert stains in the tissue nodules and areas of infiltration. Investigation of the status of myelin revealed focal areas of destruction which, however, were not as widespread as might have been expected from the severity and extent of the inflammatory involvement. Axis-cylinders were better preserved in these areas than was the myelin. The fulminating course of the disease, however, prevented a full evaluation of the loss of the myelin.

The genus *Listerella* is responsible for a wide variety of infections in various domestic animals, including encephalitis as well as other tissue localizations. Encephalitis of sheep has been most commonly discussed. The status of myelin in such cases is problematic. It is claimed that the organism is responsible for some human infections. The literature is extensive and not entirely relevant to the present

47. King, L. S.: *Am. J. Path.* **16**:467, 1940.

48. TenBroeck, C., and Seastone, C. V.: Unpublished data; cited by King.⁴⁷

discussion. A satisfactory review has been published by Graham, Levine and Morrill,⁴⁹ and numerous articles regarding human and animal infections are cited by King.⁴⁷ A curious feature is that although in cases of animal encephalitis and encephalomyelitis the causative role of *Listerella* cannot be doubted, nevertheless Koch's postulates have not been fulfilled. Organisms can be demonstrated within the involved brain tissue, but pure cultures do not produce the natural disease. Fatal meningitis is readily produced but the characteristic disseminated parenchymatous involvement seen in the natural diseases has not been reproduced experimentally.

The significance of this condition is twofold. Loss of myelin is demonstrated in an acute natural infection, although the amount and the severity of the loss are not great and demyelination is not the ruling pathologic feature. Further, the damage of myelin is related directly to the primary inflammatory process and appears to be due to the direct localization of the infecting agent. These points will be considered in more detail in the comment.

MISCELLANEOUS OBSERVATIONS

A wide variety of diseases of the nervous system has been described as occurring spontaneously in lower animals. Many have been well studied; others, only sketchily described. Unfortunately, only rarely in studies on brain diseases in animals has the problem of the destruction of myelin received attention.

In our survey of the literature, we have encountered a few reports in which data are incomplete and which do not admit of any discussion. The following instances, however, deserve mention.

Scherer⁷ mentioned briefly a demyelinating condition which he observed in bears but did not describe the disease at adequate length. In 2 animals he observed disseminated focal encephalomyelitis. There was absence of "diffuse inflammation," but small foci of lymphocytic perivascular inflammation, with loose macroglial and microglial proliferation, were noted. The lesions did not respect any boundaries between gray matter and white matter. Demyelination is mentioned as incomplete, but adequate details are not given.

Hamerton⁶⁵ described a middle-aged leopard with gradually increasing spastic paralysis of the hindlegs, becoming flaccid shortly before death. In the spinal cord, there was degeneration of pyramidal and cerebellar tracts and of the column of Goll, and the myelin was replaced by scarlet-staining globules of fat. There were disappearance of axis-cylinders, with neuroglial replacement, and "hardly any

49. Graham, R.; Levine, N. D., and Morrill, C. C.: *Listerellosis in Domestic Animals*, Bulletin 499, University of Illinois, Agricultural Experiment Station, 1943.

inflammatory reaction." He could not define the disease in terms of human pathology. His paper gives convincing illustrations.

In 1946 Hamerton⁵⁰ noted a tiger bittern which died of encephalitis. The brain showed "round cell infiltration along the course of the cerebral vessels and areas of demyelination in the intervening tissue." Further data are not presented.

COMMENT

Meaning of "Demyelinating Disease."—From the standpoint of nosology it is perhaps unfortunate that the myelin stain was ever devised. With its use, investigators can make magnificent histologic preparations exhibiting loss of myelin in a wide variety of different conditions. But emphasis has been placed on histologic details rather than on disease processes. If we wish to be literal, any disorder in which myelin is lost is a "demyelinizing disease." Yet common usage revolts at such catholicity and insists that certain diseases showing loss of myelin be categorized differently from others in which the destruction of myelin is also a prominent feature. With literal interpretation, using a myelin stain as the criterion, we should have to include such diverse states as wallerian degeneration, amyotrophic lateral sclerosis, brain abscess, Marchiafava disease and experimental cyanide poisoning, to name but a few.

It is desirable to distinguish between demyelination as a condition and demyelination as a disease. In the former the loss of myelin is secondary to some other pathogenic features. Thus, if cell bodies are first attacked, there results a loss of associated nerve fibers as a secondary phenomenon. By a similar pathogenesis interruption of axons leads to loss of the distal segments. Again, any destructive process which attacks white matter merely as an accident of location should also be excluded from "demyelinating diseases." The localization of an embolus, a thrombus, staphylococci or tubercle bacilli may be such as to produce widespread destruction of white matter. No one would call an infarct, an abscess, a traumatic cicatrix or a tuberculoma a "demyelinating disease," no matter how beautiful the Weigert stain of sections. In these instances the pathogenic factor attacks the white matter merely accidentally, by virtue of fortuitous localization, not by virtue of any essential relationship borne by myelin as such.

If one wishes to use the term "demyelinating disease," the characterizing feature by contrast is that the disease process attacks primarily and essentially the white matter of the brain. The degree of preservation of axis-cylinders is in no sense fundamental. In most of the commonly accepted "demyelinating diseases" axis-cylinders may be well

50. Hamerton, A. E.: Proc. Zool. Soc., London **116**:611, 1946.

preserved in some areas and sharply reduced in others, while in still others they may be as completely absent as are the myelin sheaths. In some zones, indeed, there may be complete destruction of all tissue. So long as the damage primarily involves white matter, or the components of white matter, the condition may be designated as a demyelinating disease. Gray matter may be incidentally affected, as in some plaques of multiple sclerosis, but the fundamental nature of the disease is not thereby altered.

DISSEMINATED ENCEPHALOMYELITIS

The whole subject of disseminated encephalomyelitis has been rather confused. The term is used in two different senses, one indicative of inflammatory involvement of brain parenchyma, the other stressing the occurrence of demyelination. This partial dichotomy and partial overlap must be considered highly illogical. The term has, of course, a literal meaning, which is only an inflammation of brain and spinal cord that is more or less focal rather than diffuse.

In the strict sense, any fundamental distinction between "encephalitis" and "encephalomyelitis" is untenable. Both represent inflammatory processes, one involving the cerebrum alone, the other the spinal cord in addition. The difficulty has arisen in human neuropathology in the attempt to use morphologic criteria to determine the etiologic explanation. Thus, in common usage "encephalitis" implies some sort of infectious agent, while "encephalomyelitis," especially when qualified by the word "disseminated," tends to suggest a non-infectious or at least an unknown agent. In the latter category demyelination may be a prominent feature, since demyelination and inflammation may go hand in hand in some acute or subacute diseases.

Such a distinction is illogical and could readily be avoided by dealing in etiologic rather than in purely morphologic terms. The known infections of the nervous system are numerous, with quite varied manifestations. Protozoa, spirochetes, bacteria and viruses, not to mention some metazoa, can involve the brain and the spinal cord. There is no need for surprise that different viruses or different bacteria produce different histologic responses in the brain—any more than in regard to other tissues. The attack may be spotty and focal or it may be relatively diffuse. In the face of known causes the subdividing of encephalitis into "types" on morphologic grounds appears to be pure supererogation.

The difficulty arises in those inflammatory conditions the causes of which are not known. This difficulty has been compounded and aggravated by attempts to deduce the causes from morphologic changes alone. Fortunately modern neuropathology has successfully emerged from the era of interminable discussion regarding "inflammation" and

"degeneration," in which "inflammation" was somehow considered synonymous with "infection." When this was recognized as untenable, the concept of "symptomatic inflammation" was introduced. The unfortunate terms obscure the real value of the concepts.

We know that the inflammatory reaction in general is a more or less specific chemotactic response, the nature of the response being determined by the character of the chemotactic agents. These agents may be of exogenous nature, such as living infectious organisms, dead organisms or the chemical fractions of such organisms; or they may be endogenous, such as factors derived from breakdown or other injury of tissues, as in burns or trauma. Various allergic manifestations, such as the Arthus or the Shwartzman phenomenon, or the hypersensitivity leading to periarteritis nodosa, can provide the necessary chemotactic stimuli for the inflammatory response. In infectious processes the response is generally determined by both exogenous and endogenous factors. In noninfectious inflammation the endogenous factors alone are responsible.

Inflammation takes many forms, because of the enormous complexity of the constituents of tissues and of the products of their decomposition, as well as the multiplicity of exogenous agents. The subject has been widely studied in general terms, but little has been done in specific reference to the nervous system. In the brain two types of inflammation may be distinguished: One is the so-called perivascular infiltration, in which infiltrating cells seem restricted to the sheaths of blood vessels; the other, the parenchymatous involvement, with infiltration of the parenchyma. The factors responsible for these two types are not known. Concerning parenchymatous involvement, the term "inflammation" cannot, of course, be restricted merely to the presence of lymphocytes, polymorphonuclear leukocytes or plasmacytes. The mobilization of microglia cells and other histiocytic elements, producing the familiar glial nodules and other manifestations, forms a nerve tissue counterpart of the granulomatous inflammation occurring in other organs.

The relation of inflammation and demyelination is complex. In most inflammatory conditions of the nervous system due to infectious agents, demyelination is not present. Translated into familiar pathologic concepts, this means that the inflammation is not severe enough to cause destruction of tissue. Occasional uncomplicated exceptions may occur. Loss of myelin in goats, discussed on a foregoing page, is a case in point. In equine encephalomyelitis, in which we have had extensive experience with the experimental although not with the natural disease, the inflammatory changes rarely destroy myelin. Yet we have Weigert preparations of guinea pig brains in which exquisite foci of loss of myelin are occasionally evident. The time factor and the

conditions of the experiments are such as to allow only one interpretation: The direct action of the infective agent is sufficiently intense to destroy the white matter at the particular sites of virus localization. A similar explanation would apply to the "demyelination" described by Farber and associates⁵¹ in human cases. In other words, the direct localization of the virus or the bacteria and the inflammatory response caused thereby destroyed the myelin.

Far different may be the explanation in disseminated encephalomyelitis of the dog. In some instances with demyelination the immediate presence of a virus in the brain has been proved, and it is quite possible that the mechanism is exactly comparable to that of equine encephalomyelitis. But there is no warrant for assuming that demyelination occurring in the dog is so caused in all instances. A different mechanism may be at work. Possibly in some foci in some animals the destruction may be primary and the inflammatory response relatively secondary. In other words, the particular focus of demyelination (i. e., of destruction of tissue) may be on a purely endogenous basis, although other inflammatory lesions in the same brain may possibly be due to direct localization of virus. Hurst³⁶ combated this concept, but his objections do not appear to us conclusive.

Thus, except under rigorously controlled conditions, the presence of a virus or other infectious agent does not prove that all demyelination present was directly caused by that virus. In the guinea pigs mentioned in a foregoing paragraph, as well as in the *Listerella* infection of goats discussed previously, it appears quite certain that direct action was responsible. But in most cases of the canine disease it is possible that the loss of myelin means destruction of tissue due to "allergic" or other endogenous factors. Any virus present may be an inciting or "sensitizing" factor, as well as a directly acting agent.

It is our personal belief that disseminated encephalomyelitis of the dog does not represent a single disease entity, unlike the demyelinations in monkey, sheep, horse and possibly moose. Instead, dogs exhibit widespread inflammatory lesions, some of which are caused by virus, some of which show demyelination and some of which are not caused by direct action of virus. The interrelations of these three variables are obscure. The term "disseminated encephalomyelitis" applies only to the inflammatory component, however produced, and is in no sense specific.

In regard to a classification of demyelinating diseases of animals, the disease described in dogs and goats is not in the same category as the one previously discussed. So far as demyelination in the dog or the goat

51. Farber, S.; Hill, A.; Connerly, M. C., and Dingle, J. H.: *J. A. M. A.* **114**: 1725, 1940.

is due to the direct local action of the virus or the bacterium, the disease is strictly analogous to a brain abscess, a tuberculoma or an infarct. That is, the loss of myelin is due to fortuitous localization of the pathogenic agent, with attendant destruction of tissue. But so far as the destruction of white matter is of purely endogenous origin (for the sake of argument, let us say it is possibly due to an "allergic" or a "sensitivity" reaction) with intrinsic predilection for white matter, the disease can be included among the true spontaneous demyelinating diseases.

CRITIQUE OF "DEMYELINATING DISEASES"

The term "demyelinating disease" has been defined, and the designated condition separated from the other conditions in which damage of white matter is also a feature. It may well be questioned whether any such distinction is truly valid, or whether it is essentially artificial.

In the study of the animal diseases discussed in the foregoing pages the strict specificity of the various conditions is impressive. Whereas in human so-called "demyelinating diseases" the nosologic outlines are vague, and by judicious selection of "transitional cases" a pseudounity may be thrown over the whole field, in lower animals such unification is not reasonable. The leukoencephalitis of horses has no counterpart in other species. In fact, the known causative agent does not act on cattle or other animals. Copper deficiency is not known to affect myelin in any species but sheep. Demyelination in the monkey bears no relation to the demyelinating disease of the dog.

An analogy may be drawn with general medicine by comparing demyelinating conditions with fevers. Fevers form an integral part of many disease pictures. In many conditions, such as pneumonia or tuberculosis, there are other equally or more important physical signs by which the disease entity is characterized. The fever is present but is merely an aspect of the total picture. To this type of fever we would compare the destruction of white matter following trauma or abscess.

On the other hand, there are many fevers unaccompanied with other physical signs, which cannot be correlated with other factors and for which no adequate explanation can be given. These, in despair, are known simply as "pyrexia of unknown origin." In such cases comparison may be drawn with "demyelinating disease" of unknown cause, such as that of the monkey.

A still different category would be fever following, say, heat stroke, in which the fever is the principal manifestation of the disease, and the "cause" is known. Comparison may be made with a primary or essential demyelinating disease the "cause" of which is known, even though the mechanisms are not. In the present state of knowledge the moldy corn disease or sway-back would fit this category.

We believe this analogy to be valid and to be in line with the opinions of Innes^{15e} and of Hurst, Cooke and Melvin³⁶ that demyelination is not a specific process due to a single cause but represents a type of response of white matter exposed to noxious stimuli.

In terms of the analogy it would be wise to study fever either as a mechanism or as a specific disease, but not febrile diseases as a whole. In animals the specificity of the individual conditions is such that any one disease may easily be studied by itself. Animal diseases also allow for ready experimentation.

The other aspect, demyelination as a mechanism, has not received sufficient attention. We assume that destruction of myelin or, preferably, damage of white matter, represents a degrading of lipids, proteins, lipoproteins and other constituents by enzymatic action, with definite specificity of both enzymes and substrates. It is not to be wondered that an enzyme system of the horse should differ from that of the sheep. It is not enough, in lower animals, to study "demyelination" in general. The process is different in each animal, and each species presents problems of its own.

Within a given species, however, there is no indication that various forms of myelin loss, or damage of white matter, are mediated by the same enzymatic processes. Only horses are subject to "moldy corn disease." But horse brain shows destruction of white matter in the course of abscess, trauma or secondary degeneration. The assumption that the disintegration of myelin is chemically the same in these various conditions is purely gratuitous. A priori considerations would lead one to expect very different histochemical reactions if the damage were caused by moldy corn or by infection or by trauma.

Unfortunately, in the past the methods of studying chemical reactions of the brain have been limited, and histochemistry has been a backward subject in neuropathology. There have been various staining reactions and histologic sequences which have engaged attention, and which have been used with some success, especially in the study of myelin loss in secondary degeneration. But the methods are relatively crude. Fortunately, in modern anatomy and general pathology, newer and more exact histochemical technics are being elaborated, with stress on unfixed material prepared by freezing-drying technics, ultraviolet absorption curves, employment of purified enzymes, solubility studies and the like. Much valuable work has already been done on the nervous system, and it is only a matter of time before these technics are elaborated and gain wider acceptance.

It is hoped that the use of the newer technics and the analysis of variations of the process of demyelination will throw more light on those individual diseases in which damage to white matter is either a minor or a major component.

SUMMARY

In this critique a demyelinating disease is defined as one in which the disease process attacks primarily and essentially the white matter of the brain. Using such a criterion one finds several important diseases of lower animals which appear to be discrete nosologic entities. In monkeys and apes a condition has been observed, of subacute to chronic course, characterized by demyelination affecting brain, optic nerve and tracts, and spinal cord. No infectious or transmissible agent has ever been demonstrated. Dietary or other environmental factors are suggested as causal, but proof is lacking.

Through the work of Innes and of Bennetts, a disease of fetal and young lambs has been well defined. Demyelination and, in severe cases, softening leading to cavitation form a distinct disease entity. There is overwhelming evidence that copper deficiency of the pregnant ewe plays a role in the etiology and that the disease may be prevented by supplementary copper feedings. But the mechanisms at work are not known.

In horses a destructive process affecting the white matter of the cerebral hemispheres, of fulminating character, has been studied. Etiologically it has been definitely traced to the ingestion of moldy corn and appears to be of "toxic" nature.

In moose of both Maine and Minnesota demyelination of spotty character has been observed, with well defined glial scars appearing in the white matter of the cerebral hemispheres. The clinical syndrome is not clearcut, no transmissible agent has been found and the cause is obscure.

Encephalomyelitis of dogs and of goats has been described, with variable destruction of white matter. This damage is in part caused by localization of infectious agents (*Listerella* in goats, distemper virus or some virus like it in dogs). But in some dogs no infectious agents can be traced, and it appears unquestionable that in the dog more than one mechanism is at work to cause the damage of the white matter.

The importance of destruction of white matter as a process, rather than as a disease, is stressed.

Notes and News

Meetings.—The first annual meeting of the International Cancer Research Commission, formed in St. Louis in 1947, has been tentatively scheduled for July 15 to 22, 1949, in Paris, France. Further information may be obtained from Ignacio Millan, chairman, Avenida Veracruz 69, México, D. F. The Commission, which originally consisted of one representative from each of forty nations, has since been joined by representatives of Finland, Iceland, Israel and New Zealand.

Transactions of the Fourth International Cancer Research Congress (Sept. 2 to 7, 1947) are being published in five parts, according to an announcement by E. V. Cowdry, president of the Congress. The first part, consisting of 267 pages, including illustrations, tables and brief summaries of all papers in English, French, German, Italian, Russian and Spanish, appeared in 1948 as no. 1, vol. 6 of ACTA (Union Internationale Contre le Cancer). The other parts of the transactions will soon be available. Cost of the complete set will be \$25 (\$5 for each part), and postal money orders for subscriptions should be sent to J. H. Maisin, editor, 61 Voer des Capucins, Louvain, Belgium. Reprints of the separate papers can also be obtained from him.

The Second Inter-American Congress on Brucellosis will be held in Washington, D. C. in October 1950.

Deaths.—William Augustus Evans, pathologist and formerly health commissioner of Chicago, died at Muldon, Miss., Nov. 8, 1948, aged 83, of heart disease. He received his medical degree from Tulane University of Louisiana, New Orleans, in 1885, and moved to Chicago, where from 1891 on he was demonstrator of pathology, and from 1895 to 1908 professor, at the College of Physicians and Surgeons of Chicago, now the school of medicine of the University of Illinois. In 1908 he joined the faculty of Northwestern University Medical School, where at the time of his death he held the title of professor of public health emeritus. In 1907 he was appointed health commissioner of Chicago, serving until 1911, when he became health editor of the *Chicago Daily Tribune*, where for twenty-three years he conducted the column on "How to Keep Well." Among his honorary degrees was D.P.H. (doctor of public health) from the University of Michigan, Ann Arbor, in 1911. Dr. Evans presented his home town of Aberdeen, Miss., with a library of some 15,000 volumes and 10,000 manuscripts as a memorial to the Evans family, which for generations lived in that section of the state. He also restored the old home of Jefferson Davis, "Beauvoir," in Biloxi, Miss., converting it into a public building now maintained by a Davis memorial association.

Gustave Ricker, for many years director of the Pathologic Institute in Magdeburg, Germany, and one of the outstanding and original European pathologists of the first half of this century, died at the age of 77 in Dresden, on Sept. 23, 1948. His famous book on the pathology of relations¹ was published in 1924. Ricker

1. Ricker, G.: Pathologie als Naturwissenschaft, Relationspathologie; für Pathologen, Physiologen, Mediziner und Biologen, Berlin, J. Springer, 1924.

postulated that all stimuli act primarily on the nervous system, and that all physiologic and pathologic responses in tissues and organs are dependent on changes of the blood and lymph circulation which are under nervous control. His experiments in which the circulation of the pancreatic region of the rabbit was studied under various degrees of irritation resulted in the establishment of his widely known *Stufengesetz* (law of degrees) and furnished a foundation for the understanding of many functional disturbances of the circulation and their sequelae. While Ricker was an outsider for a long period, frowned on by numerous advocates of cellular pathology, much of his theory has been more and more accepted during the last two decades. His doctrines will stimulate further discussion and research for many years to come.—KARL T. NEUBUERGER.

INDEX TO VOLUME 46

Subject entries are made for all articles. Author entries are made for original articles. Book Reviews and Obituaries are indexed under these headings in alphabetical order under the letters B and O, respectively.

- Abdomen:** See Gastrointestinal Tract; Peritoneum; etc.
- Abnormalities and Deformities:** See under names of organs and regions, as Heart; etc.
- Abscess:** See under names of organs and regions
- Accidents; significance of agonal changes in human liver, 132**
- Acid, Amino:** See Amino Acids
- Folic:** See Vitamins, B
- Nucleic:** See Nucleins
- Acidification:** See Tissue
- Adamantinoma and teratoma, limitation of concepts of, 256**
- Addison's Disease, 308**
- Adenocarcinoma; carcinoma of rete testis, 239**
- multicystic (Sertoli cell carcinoma), 236**
- Adenoepithelioma; polymorphism of chromophobe adenoma (adenoepithelioma) of pituitary gland, 250**
- Adenohypophysis:** See Pituitary Body
- Adenoma, acidophilic (oligochromic), exhaustion of, 245**
- bronchial, producing "alveolar cell carcinoma" pattern, 529**
- of parotid gland, 187**
- tubular, of testes, 234**
- Adipose Tissue:** See Fat; Lipoma
- Adolescence, Ewing's endothelial myeloma of; report of 2 fatal cases, 68**
- Adrenal Preparations; cholesterol of human adrenal gland; its significance in relation to adrenal function and structure, 451**
- experimental atherosclerosis; effect of desoxycorticosterone acetate on cholesterol content of blood, aorta and liver of rabbits, 536**
- Adrenals; adrenal cortex rest tumors of testes, 233**
- cholesterol of human adrenal gland; its significance in relation to adrenal function and structure, 451**
- Age; aging processes in ovaries of mice belonging to strains differing in incidence of mammary carcinoma, 401**
- Agglutinins and Agglutination:** See also Erythroblastosis, Fetal; etc.
- specific gravity of blood corpuscle; its possible significance in atherosclerosis, 97**
- Agonal Period; significance of agonal changes in human liver, 132**
- Alkalosis; absence of renal lesions in rats receiving synthetic diet low in protein, 398**
- Alveoli:** See Lungs
- Amidon, E. L.: Diffuse plasma cell myelomatosis, 183**
- Amino Acids:** See also Proteins
- effects of folic acid antagonists inoculated in embryonated eggs, 441**
- Amputation, genesis of gangrenous and reparative processes in trench foot, 1**
- Amromin, G. D.: Medionecrosis of aorta, 380**
- Anemia, splenic; quantitative approach to study of splenomegaly, 320**
- Aneurysm; medionecrosis of aorta, 380**
- Angiectasis:** See Meninges
- Angioma; diffuse angiectasis of cerebral meninges of newborn infant; report of 3 cases, 87**
- Animals:** See also Dogs; etc.
- spontaneous demyelinating diseases of animals; study in comparative pathology, 567**
- Anomalies:** See under names of organs and regions, as Heart; etc.
- Anoxemia:** See Blood, oxygen
- Anoxia:** See Blood, oxygen
- Antigens and Antibodies:** See Agglutinins and Agglutination; Immunity; Lipoids; and under specific antigens and reactions
- Antiserum:** See under names of various diseases
- Aorta, Aneurysm:** See under Aneurysm
- Embolism:** See Embolism
- experimental atherosclerosis; effect of desoxycorticosterone acetate on cholesterol content of blood, aorta and liver of rabbits, 536**
- medionecrosis of, 380**
- mitotic activity in aortic lesions of experimental cholesterol atherosclerosis of rabbits, 179**
- Appendix, human, argentaffin cells; comparative study of results obtained with modified Schmorl and Masson technics, 83**
- Appointments, 287, 501**
- Archeoblastoma, solitary suprasellar cyst of pituitary gland, 254**
- Argentaffin Cells:** See Appendix
- Armed Forces Personnel:** See also Military Medicine; etc.
- genesis of gangrenous and reparative processes in trench foot, 1**
- Armies:** See Military Medicine
- Arrhenoblastoma of testes, 230**
- Arteriosclerosis; experimental atherosclerosis; effect of desoxycorticosterone acetate on cholesterol content of blood, aorta and liver of rabbits, 536**
- medionecrosis of aorta, 380**
- mitotic activity in aortic lesions of experimental cholesterol atherosclerosis of rabbits, 179**
- specific gravity of blood corpuscle; its possible significance in atherosclerosis, 97**
- Arthritis:** See also under names of joints
- rheumatoid; chronic inflammatory lesions of skeletal muscle in rheumatoid arthritis and in other diseases, 301**
- rheumatoid, visceral lesions in case of, 59**
- Spinal:** See Spine, arthritis
- Aschoff Body:** See Rheumatic Fever
- Atheroma:** See Arteriosclerosis
- Atherosclerosis:** See Arteriosclerosis
- Atrophy, muscular, 309**
- Autopsies; postmortem examination of teeth and supporting structures to aid in personal identification, 119**
- significance of agonal changes in human liver, 132**
- Avitaminosis:** See under Vitamins
- Awards, 287**
- Bacilli:** See Bacteria
- Bacteria:** See under Viruses; etc.
- Lepra:** See Leprosy
- Baker, A. B.: Interrelationship of diseases of liver and brain, 268**
- Banti's Disease:** See Anemia, splenic
- Barron, S. S.: Significance of beta granules in islets of Langerhans of pancreas, 159**
- Basedow's Disease:** See Goiter, exophthalmic
- Basophilism:** See under Pituitary Body
- Basophils:** See Pituitary Body
- Besnier-Bocock's Disease:** See Sarcoidosis

- Beta Granules: See Cells
 Biliary Tract: See Liver
 Biopsies of bone marrow of experimental animals, method for, 498
 significance of agonal changes in human liver, 132
 Black, C. E.: Influence of local acidification of tissue bordering cancerous growths; with special reference to eosinophil, paneth cell and acidophilic plasma cell, 107
 Blanco, F. L.: Silvering of *lepra bacilli* in tissues, 542
 Block, M.: Effect of nitrogen mustard in mycosis fungoides, 519
 Genesis of gangrenous and reparative processes in trench foot, 1
 Blood: See also Erythrocytes; Infants, newborn; Leukocytes; and under diseases, organs and regions; etc.
 cells; specific gravity of blood corpuscle; its possible significance in atherosclerosis, 97
 cholesterol; experimental atherosclerosis; effect of desoxycorticosterone acetate on cholesterol content of blood, aorta and liver of rabbits, 536
 Diseases: See Anemia; Hemopoietic System; Leukemia; etc.
 fats and lipoids; mitotic activity in aortic lesions of experimental cholesterol atherosclerosis of rabbits, 179
 fats and lipoids; specific gravity of blood corpuscle; its possible significance in atherosclerosis, 97
 oxygen; viral versus toxic hepatic necrosis, 358
 pressure; medionecrosis of aorta, 380
 Vessels: See also Cardiovascular System; Periarthritis; etc.
 vessels; aging processes in ovaries of mice belonging to strains differing in incidence of mammary carcinoma, 401
 Boeck's Sarcoid: See Sarcoidosis
 Bones: See also under names of bones
 dyschondroplasia with hemangiomatosis (Maffucci's syndrome) and teratoid tumor of ovary, 74
 Ewing's endothelial myeloma of adolescents; report of 2 fatal cases, 68
 marrow; cytologic changes in bronchogenic carcinoma following treatment with nitrogen mustard (methyl-bis β -chloroethyl amine), 503
 marrow; effects of folic acid deficiency and folic acid antagonist on chicks, 559
 marrow, of experimental animals; method for biopsy, 498
 reticulum cell sarcoma, 467
 BOOK REVIEWS:
 Bronchogenic Carcinoma and Adenoma, with Chapter on Mediastinal Tumors; B. M. Fried, 400
 Cancer: Carcinogenesis, Carcinoreistencia, Carcinoinhibicao; M. Mosinger, 86
 Education for Professional Responsibility: Report of Proceedings of "Inter-Professions Conference on Education for Professional Responsibility," 190
 Hematology; C. C. Sturges, 502
 Hetero-Specific Alteration Therapy: New Treatment for Pulmonary Tuberculosis Based on Specific Cellular Alteration Produced by a Mixed Autolysate of Typhoid Bacilli and Gonococci; S. Nukada and C. Ryu, 85
 Histopathology of Peripheral and Central Nervous Systems; G. B. Hassin, 400
 Medical Writing: Technique and Art; M. Fishbein, 190
 Neurosurgical Pathology; I. M. Scheinker, 502
 Book Reviews--Continued
 Outline of Histology; M. M. Hoskins, 190
 Rh Blood Groups and Their Clinical Effects; P. L. Mollison, A. E. Mourant and R. R. Race, 288
 Books received, 85, 190, 288, 400, 502
 Boullaud's Disease: See Rheumatic Fever
 Brain: See also Meninges; Nervous System; etc.
 diffuse angiectasis of cerebral meninges of newborn infant; report of 3 cases, 87
 intracranial vascular lesions in late rheumatic heart disease, 191
 spontaneous demyelinating diseases of animals; study in comparative pathology, 567
 Breast, cancer, aging processes in ovaries of mice belonging to strains differing in incidence of, 401
 inflammation; plasma cell mastitis, 313
 mammary lipoma, 386
 Bright's Disease: See Nephritis
 Bronchi, adenoma producing "alveolar cell carcinoma" pattern, 529
 carcinoma; cytologic changes following treatment with nitrogen mustard (methyl-bis β -chloroethyl amine), 503
 Bruger, M.: Experimental atherosclerosis; effect of desoxycorticosterone acetate on cholesterol content of blood, aorta and liver of rabbits, 536
 Bryan, A. L.: Comparison of thymic hyperplasia in myasthenia gravis and exophthalmic goiter, 212
 Cadavers: See Autopsies
 Calcium and Calcium Compounds; effect of sodium chloride deprivation on growing rat, 260
 Cancer: See also Sarcoma; Tumors; and under names of organs and regions, as Breast; Bronchi; Gastrointestinal Tract; Meninges; Reticuloendothelial System; Testes; etc.
 cells; bronchial adenoma producing "alveolar cell carcinoma" pattern, 529
 research, official organ of American Association for, 287
 Canines: See Dogs
 Carbuncle, Renal: See Nephritis
 Carcinoma: See Cancer
 Cardiovascular Diseases: See also Blood vessels; Heart
 cholesterol of human adrenal gland; its significance in relation to adrenal function and structure, 451
 intracranial vascular lesions in late rheumatic heart disease, 191
 Cardiovascular System: See also Blood vessels; Heart; etc.
 paradoxical embolism; review of literature, with report of case in which this condition followed administration of "dicumarol," 43
 Carcinogens: See Cancer
 Cecum; fatal viral hepatitis complicated by phlegmonous cecitis and ileocecal intussusception, 493
 Cells: See also Blood, cells; Cancer, cells; Phagocytes and Phagocytosis; Tissue; etc.
 alveolar; bronchial adenoma producing "alveolar cell carcinoma" pattern, 529
 cytologic changes in bronchogenic carcinoma following treatment with nitrogen mustard (methyl-bis β -chloroethyl amine), 503
 diffuse plasma cell myelomatosis, 183
 division; mitotic activity in aortic lesions of experimental cholesterol atherosclerosis of rabbits, 179
 division; nucleic acids and cytologic changes in regenerating rat liver, 164
 effect of nitrogen mustard in mycosis fungoides, 519

Cells—Continued

- influence of local acidification of tissue bordering cancerous growths with reference to eosinophil, paneth cell and acidophilic plasma cell, 107
- neoplastic diseases of dogs; mast cell sarcoma, lymphosarcoma, histiocytoma, 477
- nucleic acids and cytologic changes in regenerating rat liver, 164
- Permeability: See Osmosis and Permeability
- plasma cell mastitis, 313
- significance of beta granules in islets of Langerhans of pancreas, 159
- Cerebrum: See Brain
- Cheek, J. H.: Bronchial adenoma producing "alveolar cell carcinoma" pattern, 529
- Chemotherapy: See under names of diseases and chemotherapeutic agents
- Chemotropism: See Eosinophils; Leukocytes
- Children: See also Infants
- dyschondroplasia with hemangiomatosis (Maffucci's syndrome) and teratoid tumor of ovary, 74
- Chlorides; effect of sodium chloride deprivation on growing rat, 260
- Chloroethylamines; effect of nitrogen mustard in mycosis fungoides, 519
- cytologic changes in bronchogenic carcinoma following treatment with nitrogen mustard (methyl-bis β -chloroethyl amine), 503
- Cholesterol: See also Lipoids
- in Blood: See Blood, cholesterol
- mitotic activity in aortic lesions of experimental cholesterol atherosclerosis of rabbits, 179
- of human adrenal gland; its significance in relation to adrenal function and structure, 451
- Circulatory System: See Blood, circulation; Blood, vessels; Cardiovascular System; Heart
- Cirrhosis: See Liver
- Clagett, O. T.: Comparison of thymic hyperplasia in myasthenia gravis and exophthalmic goiter, 212
- Coccidiosis, hepatic; control in rabbits with succinylsulfathiazole U. S. P.; study of mode of action of sulfonamides, 128
- Cold; genesis of gangrenous and reparative processes in trench foot, 1
- Colon: See Cecum; Gastrointestinal Tract; Intestines
- Colvin, S. H., Jr.: Adenoma of parotid gland, 187
- Communicable Diseases: See Immunity; etc.
- Congress: See Societies
- Conjunctiva; effect of sodium chloride deprivation on growing rat, 260
- Copper, deficiency; spontaneous demyelinating diseases of animals; study in comparative pathology, 567
- Corpus Luteum; aging processes in ovaries of mice belonging to strains differing in incidence of mammary carcinoma, 401
- Correction in obituary of Prof. C. Bonne (Arch. Path. 45:795 [June] 1948), 288
- Cortin: See Adrenal Preparations
- Costero, I.: Some problems related to origin and meaning of pituitary gland tumors, 243
- Cragan, M. D.: Myocardial changes in poliomyelitis, 202
- Cramer, O. S.: Paradoxical embolism; review of literature, with report of case in which this condition followed administration of "dicumarol," 43
- Cranium; intracranial vascular lesions in late rheumatic heart disease, 191
- Cutlino, J. T.: Effect of sodium chloride deprivation on growing rat, 260
- Cysts: See under names of organs and regions, as Spleen; etc.
- Cytology: See Cells
- Cytoplasm: See Protoplasm
- Dark-Field Illumination: See Viruses
- Death: See also Obituaries
- sudden; sarcoidosis involving heart; report of case, 289
- sudden; significance of agonal changes in human liver, 132
- Deceleration: See Accidents
- Deformities: See under names of organs and regions
- Degeneration, hyaline; aging processes in ovaries of mice belonging to strains differing in incidence of mammary carcinoma, 401
- Denst, J.: Intracranial vascular lesions in late rheumatic heart disease, 191
- Dentures: See Teeth
- Derbyshire, R. C.: Paradoxical embolism; review of literature, with report of case in which this condition followed administration of "dicumarol," 43
- Derlinger, M. K.: Hereditary renal disease and amyloidosis in mice, 49
- Dermatomyositis, 305
- Desoxycorticosterone Acetate: See Adrenal Preparations
- Dicumarol; paradoxical embolism; review of literature, with report of case in which this condition followed administration of "dicumarol," 43
- Diet and Dietetics: See also Vitamins; Nutrition; and under names of foods
- absence of renal lesions in rats receiving synthetic diet low in protein, 398
- effect of sodium chloride deprivation on growing rat, 260
- effects of folic acid deficiency and folic acid antagonist on chicks, 559
- significance of beta granules in islets of Langerhans of pancreas, 159
- Digestive System: See Gastrointestinal Tract; Intestines; Pancreas; etc.
- Disasters; postmortem examination of teeth and supporting structures to aid in personal identification, 119
- Disease, demyelinating; spontaneous demyelinating diseases of animals; study in comparative pathology, 567
- Dogs: See also Animals
- mast cell sarcoma, lymphosarcoma, histiocytoma; neoplastic diseases of dogs, 477
- Dolgop, V. B.: Myocardial changes in poliomyelitis, 202
- Duff, G. L.: Mitotic activity in aortic lesions of experimental cholesterol atherosclerosis of rabbits, 179
- Dyes: See Stains and Staining
- Dyschondroplasia with hemangiomatosis (Maffucci's syndrome) and teratoid tumor of ovary, 74
- Dysplasia, Mesodermal: See Mesoderm and Mesodermal Tissues
- Dystrophy, muscular, 309
- Effusions: See Exudates and Transudates
- Eggs, embryonated, effects of folic acid antagonists inoculated in, 441
- Elmeria Stiedae: See Intestines, parasites
- Embryology: See also Eggs; Ovary; Teratoma; and under organs, regions and embryologic structures
- effects of folic acid antagonists inoculated in embryonated eggs, 441
- Embolism, paradoxical; review of literature, with report of case in which this condition followed administration of "dicumarol," 43
- Encephalomyelitis; spontaneous demyelinating diseases of animals; study in comparative pathology, 567
- Endocarditis; quantitative approach to study of splenomegaly, 320
- Rheumatic: See Rheumatic Fever
- subacute bacterial, 308

- Eosinophils: See also Leukocytes
influence of local acidification of tissue bordering cancerous growths with reference to eosinophil, paneth cell and acidophilic plasma cell, 107
- Epinephrine: See Adrenal Preparations
- Epithelium, acinous; adenoma of parotid gland, 187
- Erythroblastosis, Fetal, or kernicterus, 276
- Erythroblasts: See Erythrocytes
- Erythrocytes: See also Anemia; Blood, cells; Polycythemia; etc.
specific gravity of blood corpuscle; its possible significance in atherosclerosis, 97
- Ewing Sarcoma: See under Sarcoma
- Exophthalmos: See Goiter, exophthalmic
- Exudates and Transudates: See also Peritoneum
mechanisms of leukopenia with inflammation; additional leukopenic factor found in alkaline exudates, 145
- Eyes, development of state refractory to growth of mouse tumor implanted in anterior chamber of guinea pig eye, 35
- Fasting; significance of beta granules in islets of Langerhans of pancreas, 159
- Fat: See also Lipoids
mammary lipoma, 386
necrosis; plasma cell mastitis, 313
- Felitelberg, S.: Quantitative approach to study of splenomegaly, 320
- Fetus: See Embryology
- Fever: See Rheumatic Fever
- Fito, G. L.: Silvering of lepra bacilli in tissues, 542
- Food: See Diet and Dietetics; Nutrition; Vitamins
- Foot, trench, genesis of gangrenous and reparative processes in, 1
- Franklin, M.: Viral versus toxic hepatic necrosis, 338
- Gaensler, E. A.: Cytologic changes in bronchogenic carcinoma following treatment with nitrogen mustard (methyl-bis β -chloroethyl amine), 503
- Gangrene; genesis of gangrenous and reparative processes in trench foot, 1
- Gastrointestinal Tract: See also Intestines; etc.
influence of local acidification of tissue bordering cancerous growths with reference to eosinophil, paneth cell and acidophilic plasma cell, 107
- Gaucher's Disease: See Anemia, splenic
- Genetics: See Heredity
- Gerundo, M.: Control of hepatic coccidiosis of rabbits with succinylsulfathiazole U. S. P.; study of mode of action of sulfonamides, 128
- Giffen, H. K.: Ewing's endothelial myeloma of adolescents; report of 2 fatal cases, 68
- Glia: See Neuroglia
- Globulin; mechanisms of leukopenia with inflammation; additional leukopenic factor found in alkaline exudates, 145
- Globus Pallidus: See Lenticular Nucleus
- Glycogen; significance of agonal changes in human liver, 132
- Goats: See Animals
- Godwin, J. T.: Adenoma of parotid gland, 187
- Goiter: See also Thyroid
exophthalmic, and myasthenia gravis, comparison of thymic hyperplasia in, 212
- Gonadotropic Substances: See Pituitary Body
- Gonads: See Ovary; Testes; etc.
- Gordon, A. J.: Quantitative approach to study of splenomegaly, 320
- Gordon, I.: Specific gravity of blood corpuscle; its possible significance in atherosclerosis, 97
- Granulocytes: See Leukocytes
- Granuloma; visceral lesions in case of rheumatoid arthritis, 59
- Graves' Disease: See Goiter, exophthalmic
- Gravity, specific, of blood corpuscle; its possible significance in atherosclerosis, 97
- Greco, J.: Argentaffin cells of human appendix; comparative study of results obtained with modified Schmorl and Masson techniques, 83
- Growth: See Diet and Dietetics; Embryology; Nutrition
- Gruenewald, P.: Visceral lesions in case of rheumatoid arthritis, 59
- Haipert, B.: Plasma cell mastitis, 313
- Haptens: See Immunity; Lipoids
- Heart: See also Blood, circulation; Cardiovascular System; etc.
abnormalities; diffuse angiectasis of cerebral meninges of newborn infant; report of 3 cases, 87
abnormalities; paradoxical embolism; review of literature, with report of case in which this condition followed administration of "dicumarol," 43
- Diseases: See Cardiovascular Diseases; Endocarditis; Myocarditis; etc.
myocardial changes in poliomyelitis, 202
- sarcoidosis involving; report of case with sudden death, 289
- Heid, G. J.: Hepatic heterotopy in spleen capsule, 377
- Helme-Medin Disease: See Poliomyelitis
- Hemagglutination: See Agglutinins and Agglutination
- Hemangioma: See also Angioma
dyschondroplasia with hemangiomatosis (Maffucci's syndrome) and teratoid tumor of ovary, 74
- Hematology: See Blood; Hemopoietic System
- Hematoxylin: See Stains and Staining
- Hematuria: See Nephritis
- Hemoglobin and Hemoglobin Compounds: See Anemia; Blood; Erythrocytes; etc.
- Hemolysis: See Anemia; Erythroblastosis, Fetal; Jaundice
- Hemochromatosis, 273
- Hemoclastic Crisis; mechanisms of leukopenia with inflammation; additional leukopenic factor found in alkaline exudates, 145
- Hemopoiesis: See Hemopoietic System
- Hemopoietic System: See also Leukocytes
cytologic changes in bronchogenic carcinoma following treatment with nitrogen mustard (methyl-bis β -chloroethyl amine), 503
diseases; effects of folic acid antagonists inoculated in embryonated eggs, 441
- Hemosiderin; cytologic changes in bronchogenic carcinoma following treatment with nitrogen mustard (methyl-bis β -chloroethyl amine), 503
- Hepatitis: See also Hepatitis, Infectious; Jaundice; Liver
fatal viral, complicated by phlegmonous cecitis and ileocecal intussusception, 493
- Hepatitis, Infectious; viral versus toxic hepatic necrosis, 338
- Hepar Lobatum: See Liver, cirrhosis
- Heredity; hereditary renal disease and amyloidosis in mice, 49
- Heston, W. E.: Hereditary renal disease and amyloidosis in mice, 49
- Histiocytoma; neoplastic diseases of dogs; mast cell sarcoma, lymphosarcoma, histiocytoma, 477
- HN2: See Chloroethylamines
- Holder, E. C.: Quantitative approach to study of splenomegaly, 320
- Hormones: See Adrenal Preparations; Pituitary Preparations; under names of organs, as Ovary; etc.
- Horses: See Animals
- Hunger: See Fasting
- Hydrogen Ion: See Alkalosis
- Hypercholesterolemia: See Blood, cholesterol
- Hyperplasia: See Thymus

- Hyperthyroidism:** See Thyroid, hyperthyroidism
- Hypophysis:** See Pituitary Body
- Hypothermia:** See Cold
- Icterus:** See Jaundice
- Identification, personal, postmortem examination of teeth and supporting structures to aid in, 119**
- Immunity:** See also Antigens and Antibodies; etc.
development of state refractory to growth of mouse tumor implanted in anterior chamber of guinea pig eye, 35
- Inanition:** See Fasting
- Infantile Paralysis:** See Poliomyelitis
- Infants:** See also Children
newborn; diffuse angiectasis of cerebral meninges; report of 3 cases, 87
- Infection:** See also Immunity; Viruses; and under names of bacteria
- Inflammation, mechanisms of leukopenia with; additional leukopenic factor found in alkaline exudates, 145**
- Insulin; significance of beta granules in islets of Langerhans of pancreas, 159**
- Interstitial Cells:** See Ovary
- Intervertebral Disks:** See Spine
- Intestines:** See also Gastrointestinal Tract
effects of folic acid deficiency and folic acid antagonist on chicks, 559
fatal viral hepatitis complicated by phlegmonous enteritis and ileocecal intussusception, 493
Intussusception: See Intussusception
parasites; control of hepatic coccidiosis of rabbits with succinylsulfathiazole U. S. P.; study of mode of action of sulfonamides, 128
- Intussusception, ileocecal, and phlegmonous enteritis, fatal viral hepatitis complicated by, 493**
- Islands of Langerhans:** See Pancreas
- Jaundice, Homologous Serum:** See Hepatitis, Infectious
interrelationship of diseases of liver and brain, 268
significance of agonal changes in human liver, 132
- Jaws; postmortem examination of teeth and supporting structures to aid in personal identification, 119**
- Joints:** See under names of joints
- Journals:** See Periodicals
- Kernicterus:** See Erythroblastosis, Fetal
- Ketosteroids:** See Steroids
- Khanolkar, V. R.: Reticulum cell sarcoma of bone, 467**
- Kidneys; absence of renal lesions in rats receiving synthetic diet low in protein, 398**
Diseases: See also Nephritis
diseases; cholesterol of human adrenal gland; its significance in relation to adrenal function and structure, 451
diseases; hereditary renal disease and amyloidosis in mice, 49
effect of sodium chloride deprivation on growing rat, 260
- King, J. M.: Dyschondroplasia with hemangiomatosis (Maffucci's syndrome) and teratoid tumor of ovary, 74**
- King, L. S.: Spontaneous demyelinating diseases of animals; study in comparative pathology, 567**
- Klinge-Aschoff Nodule:** See Rheumatic Fever
- Kuzma, J. F.: Dyschondroplasia with hemangiomatosis (Maffucci's syndrome) and teratoid tumor of ovary, 74**
- Laboratories; Public Health Research Institute of City of New York, 501**
virus laboratory, University of Buffalo, Buffalo General Hospital, 501
- Laennec's Disease:** See Liver, cirrhosis
- Langerhans' Islands:** See Pancreas
- Laskey, A.: Argentaffin cells of human appendix; comparative study of results obtained with modified Schmorl and Masson techniques, 83**
- Lecithins:** See Lipoids
- Leptoleukocyte Nucleus, degeneration; interrelationship of diseases of liver and brain, 268**
- Leprosy; silverying of lepra bacilli in tissues, 542**
- Leptomeninges:** See Meninges
- Letterer-Siwe Disease:** See Reticuloendothelial System
- Leukemia; diffuse plasma cell myelomatosis, 183**
quantitative approach to study of splenomegaly, 320
- Leukocidin:** See Leukocytes
- Leukocytes:** See also Eosinophils; Leukemia; Phagocytes and Phagocytosis; etc.
count; mechanisms of leukopenia with inflammation; additional leukopenic factor found in alkaline exudates, 145
effect of nitrogen mustard in mycosis fungoides, 519
specific gravity of blood corpuscle; its possible significance in atherosclerosis, 97
- Leukopenia:** See Hemoclastic Crisis
- Leukosis:** See Leukemia
- Lipemia:** See Blood, fats and lipoids
- Lipids:** See Lipoids
- Lipoblastosis:** See Fat
- Lipoidosis:** See Anemia, splenic; Lipoids
- Lipoids:** See also Cholesterol; Fat; etc.
nucleic acids and cytologic changes in regenerating rat liver, 164
- Lipoma, mammary, 386**
- Lipomatosis:** See Fat; Lipoma
- Lipophages:** See Blood, fats and lipoids; Phagocytes and Phagocytosis
- Liposarcoma; mammary lipoma, 386**
- Liver, cirrhosis; quantitative approach to study of splenomegaly, 320**
control of hepatic coccidiosis of rabbits with succinylsulfathiazole U. S. P.; study of mode of action of sulfonamides, 128
experimental atherosclerosis; effect of desoxycorticosterone acetate on cholesterol content of blood, aorta and liver of rabbits, 536
hepatic heterotopy in splenic capsule, 377
human, significance of agonal changes in, 132
regeneration; nucleic acids and cytologic changes in rat, 164
viral versus toxic hepatic necrosis, 338
- Loeb, L.: Aging processes in ovaries of mice belonging to strains differing in incidence of mammary carcinoma, 401**
- Lowenhaupt, E.: Absence of renal lesions in rats receiving synthetic diet low in protein, 398**
- Lowenstein, B. E.: Experimental atherosclerosis; effect of desoxycorticosterone acetate on cholesterol content of blood, aorta and liver of rabbits, 536**
- Lungs:** See also Bronchi; etc.
bronchial adenoma producing "alveolar cell carcinoma" pattern, 529
- Lupus erythematosus, disseminated, 395**
- Lymph Nodes:** See Lymphosarcoma; etc.
- Lymphatic System; cytologic changes in bronchogenic carcinoma following treatment with nitrogen mustard (methyl-bis β -chloroethyl amine), 503**
- Lymphoblasts:** See Lymphocytes
- Lymphocytes; effect of nitrogen mustard in mycosis fungoides, 519**
- Lymphogranuloma, Schaumann's:** See Sarcoidosis
- Lymphoid Tissue:** See Lymphatic System
- Lymphopenia:** See Lymphocytes

- Lymphosarcoma; mast cell sarcoma, lymphosarcoma, histiocytoma; neoplastic diseases of dogs, 477
of testes, 241
- Lynch, J. P.: Cytologic changes in bronchogenic carcinoma following treatment with nitrogen mustard (methyl-bis β -chloroethyl amine), 503
- McDonald, J. R.: Comparison of thymic hyperplasia in myasthenia gravis and exophthalmic goiter, 212
- McKay, D. G.: Cytologic changes in bronchogenic carcinoma following treatment with nitrogen mustard (methyl-bis β -chloroethyl amine), 503
- McKeown, C. E.: Sarcoidosis involving heart; report of case with sudden death, 289
- McMillan, G. C.: Mitotic activity in aortic lesions of experimental cholesterol atherosclerosis of rabbits, 179
- Macrophages: See Phagocytes and Phagocytosis
- Maffucci Syndrome: See Dyschondroplasia
- Malformations: See under names of organs and regions
- Malnutrition: See under Nutrition
- Mammary Gland: See Breast
- Mandible: See Jaws
- Marie-Strümpell Disease: See Spine, arthritis
- Masson Technic: See Stains and Staining
- Mast Cell: See Cells
- Mastitis: See Breast, inflammation
- Maxillary Bone: See Jaws
- Medical Societies: See Societies
- Medicine, Military: See Military Medicine
- Veterinary: See Veterinary Medicine
- Medin-Hcine Disease: See Poliomyelitis
- Medionecrosis: See Aorta
- Meehan, M. C.: Spontaneous demyelinating diseases of animals; study in comparative pathology, 567
- Megakaryocytes: See Blood, cells; Bones, marrow
- Megaloblasts: See Anemia; Bones, marrow; Erythrocytes
- Melbomian Glands: See Eyelids
- Meninges, blood supply; diffuse angiectasis of cerebral meninges of newborn infant; report of 3 cases, 87
intracranial vascular lesions in late rheumatic heart disease, 191
- Menkin, V.: Mechanisms of leukopenia with inflammation; additional leukopenic factor found in alkaline exudates, 145
- Mesenchyme: See Mesoderm and Mesodermal Tissues
- Mesoderm and Mesodermal Tissues; dyschondroplasia with hemangiomatosis (Maffucci's syndrome) and teratoid tumor of ovary, 74
- Metabolism, disorders; experimental atherosclerosis; effect of desoxycorticosterone acetate on cholesterol content of blood, aorta and liver of rabbits, 536
- Microscopy: See Stains and Staining
- Midbrain: See Brain, tumors
- Military Medicine; viral versus toxic hepatic necrosis, 338
- Mitosis: See Cells, division
- Monkeys: See Animals
- Monocytes: See Leukocytes
- Moose: See Animals
- Morgan, H. R.: Effects of folic acid antagonists inoculated in embryonated eggs, 441
- Mucicarmine: See Stains and Staining
- Mucocoele: See Appendix
- Muirhead, E. E.: Bronchial adenoma producing "alveolar cell carcinoma" pattern, 529
- Mulligan, R. M.: Neoplastic diseases of dogs; mast cell sarcoma, lymphosarcoma, histiocytoma, 477
- Murphy, J. C.: Effect of nitrogen mustard in mycosis fungoides, 519
- Muscles, Dystrophy: See Dystrophy, muscular skeletal; chronic inflammatory lesions in rheumatoid arthritis and in other diseases, 301
- Myasthenia gravis, 309
and exophthalmic goiter, comparison of thymic hyperplasia in, 212
- Mycosis fungoides, effect of nitrogen mustard in, 519
- Myelin: See Nervous System
- Myeloma; diffuse plasma cell myelomatosis, 183
Ewing's endothelial myeloma of adolescents; report of 2 fatal cases, 68
- Myocarditis; myocardial changes in poliomyelitis, 202
- Myocardium: See Heart
- Myositis; chronic inflammatory lesions of skeletal muscle in rheumatoid arthritis and in other diseases, 301
- Myxedema, 308
- Necropsies: See Autopsies
- Necrosis: See Aorta; Liver
- Neoplasms: See Cancer; Sarcoma; Tumors
- Nephritis; hereditary renal disease and amyloidosis in mice, 49
- Nephrosis: See Kidneys, diseases; Nephritis
- Nerves: See also Nervous System
miscellaneous diseases of central nervous system and peripheral nerves, 311
Tumors: See under names of tumors
- Nervous System: See also Brain; Nerves
miscellaneous diseases of central nervous system and peripheral nerves, 311
spontaneous demyelinating diseases of animals; study in comparative pathology, 567
Tumors: See under names of tumors
- Neuburger, K. T.: Intracranial vascular lesions in late rheumatic heart disease, 191
William Augustus Evans, 599
- Neuroglia; spontaneous demyelinating diseases of animals; study in comparative pathology, 567
- Neurohypophysis: See Pituitary Body
- Neutrophils: See Leukocytes
- Newborn Infants: See Infants, newborn
- Nipple: See Breast
- Nitrogen Mustards: See Chloroethylamines
- Nodes: See Arthritis, rheumatoid; Rheumatic Fever
- Nomenclature; neoplastic diseases of dogs; mast cell sarcoma, lymphosarcoma, histiocytoma, 477
- Nucleins; nucleic acids and cytologic changes in regenerating rat liver, 164
- Nutrition: See also Diet and Dietetics; Vitamins
effect of sodium chloride deprivation on growing rat, 260
- Obituaries:
Evans, W. A., 599
Ricker, Gustave, 599
Stitt, E. R., 287
- Ogle, R. S.: Influence of local acidification of tissue bordering cancerous growths with reference to eosinophil, paneth cell and acidophilic plasma cell, 107
- Ogryzlo, M. A.: Chronic inflammatory lesions of skeletal muscle in rheumatoid arthritis and in other diseases, 301
- Oleson, J. J.: Method for biopsy of bone marrow of experimental animals, 498
- Ollier's Disease: See Dyschondroplasia
- Osmosis and Permeability; influence of local acidification of tissue bordering cancerous growths with reference to eosinophil, paneth cell and acidophilic plasma cell, 107

- Ovary: See also Corpus Luteum
aging processes in ovaries of mice belonging to strains differing in incidence of mammary carcinoma, 401
dyschondroplasia with hemangiomatosis (Maffucci's syndrome) and teratoid tumor of, 74
- Ovum: See also Eggs; Embryology
- Pancreas; significance of beta granules in islets of Langerhans of, 159
Pancreatin: See under Pancreas
Paneth Cell: See Cells
Pantothenic Acid: See Vitamins, B
Paralysis, Infantile: See Poliomyelitis
Parasites: See Intestines, parasites
Parham, A. R.: Testicular tumors; interstitial cell and miscellaneous neoplasms, 229
Paris, A. S.: Effect of sodium chloride deprivation on growing rat, 260
Parker, J. M.: Plasma cell mastitis, 313
Parotid Gland, adenoma of, 187
Parotiditis nodosa, 308
Periodicals; official organ of American Association for Cancer Research, 287
Transactions of Fourth International Cancer Research Congress, 599
Peritoneum; visceral lesions in case of rheumatoid arthritis, 59
Phagocytes and Phagocytosis: See also Immunity; Leukocytes; Reticuloendothelial System
aging processes in ovaries of mice belonging to strain differing in incidence of mammary carcinoma, 401
Phosphatide: See Blood, fats and lipoids; Lipoids
Pituitary Body, tumors, some problems related to origin and meaning of, 243
Pituitary Preparations; cholesterol of human adrenal gland; its significance in relation to adrenal function and structure, 451
Plasma: See Blood
Cells: See Blood cells; Bones, marrow; Leukemia; Reticuloendothelial System
Plourea; visceral lesions in case of rheumatoid arthritis, 59
Poisons and Poisoning; viral versus toxic hepatic necrosis, 338
Poliomyelitis, myocardial changes in, 202
Polyarteritis Nodosa: See Periarteritis nodosa
Polycythemia vera; quantitative approach to study of splenomegaly, 320
Polymyositis: See Myositis
Popper, H.: Significance of agonal changes in human liver, 132
Viral versus toxic hepatic necrosis, 338
Portal Cirrhosis: See Liver, cirrhosis
Postmortems: See Autopsies
Potter, E. L.: Diffuse angiectasis of cerebral meninges of newborn infant; report of 3 cases, 87
Pregnancy: See also Fetus
Prizes: See Awards
Proteins: See also Amino Acids; etc.
absence of renal lesions in rats receiving synthetic diet low in, 398
Protoplasm: See also Cells
nucleic acids and cytologic changes in regenerating rat liver, 164
Protozoa; control of hepatic coccidiosis of rabbits with succinylsulphathiazole U.S.P.; study of mode of action of sulfonamides, 128
Refraction: See under Eyes
Respiratory Tract: See Bronchi
Reticuloendothelial System: See also Anemia, splenic; Liver; Phagocytes and Phagocytosis; Spleen; etc.
effect of nitrogen mustard in mycosis fungoides, 519
Reticuloendothelial System—Continued
Ewing's endothelial myeloma of adolescents; report of 2 fatal cases, 68
reticulum cell sarcoma of bone, 467
Reticuloendotheliomatosis: See Reticuloendothelial System
Reticuloendotheliosis: See Anemia; Leukemia; Liver; Phagocytes and Phagocytosis; Reticuloendothelial System
Reticulosis: See Reticuloendothelial System
Reticulum: See Reticuloendothelial System; Stains and Staining; Tissue
Rh Factor: See Agglutinins and Agglutination; Erythroblastosis, Fetal
Rheumatic Fever, 308
intracranial vascular lesions in late rheumatic heart disease, 191
Rheumatism, Acute: See Rheumatic Fever
Ritter, H. B.: Method for biopsy of bone marrow of experimental animals, 498
Roentgenotherapy: See under names of diseases
Rogers, W. F., Jr.: Cholesterol of human adrenal gland; its significance in relation to adrenal function and structure, 451
Rosenthal, M. H.: Effect of sodium chloride deprivation on growing rat, 260
Rous Sarcoma: See Sarcoma, Rous
Sarcoidosis involving heart; report of case with sudden death, 289
Sarcoma: See also Cancer; Liposarcoma; Lymphosarcoma; Tumors; etc.
mast cell, lymphosarcoma, histiocytoma; neoplastic diseases of dogs, 477
reticulum cell, of bone, 467
Rous; effects of folic acid deficiency and folic acid antagonist on chicks, 559
Schilling, J. A.: Development of state refractory to growth of mouse tumor implanted in anterior chamber of guinea pig eye, 35
Schlichter, J. G.: Medioncrosis of aorta, 380
Schmorl Technic: See Stains and Staining
Schools; Chicago Medical School approved, 287
Sclerosis: See Arteriosclerosis; Nephritis; Splenomegaly; etc.
Scott, T. M.: Sarcoidosis involving heart; report of case with sudden death, 289
Scully, R. E.: Testicular tumors; interstitial cell and miscellaneous neoplasms, 229
Seminoma, spermatocytic, of testes, 236
Sertoli cell carcinoma, multicystic adenocarcinoma, 236
Serum: See Blood
Immune: See Poliomyelitis
Sheep: See Animals
Silver: See Stains and Staining
Sinus, Valsalva's: See Aneurysm
Siwe-Letterer Disease: See Reticuloendothelial System
Skeleton: See under Bones
Skull: See Cranium
Snell, A. C., Jr.: Development of state refractory to growth of mouse tumor implanted in anterior chamber of guinea pig eye, 35
Societies; American Association of Pathologists and Bacteriologists, 287
American Society of Clinical Pathologists, 287
International Cancer Research Commission, 599
Second Inter-American Congress on Brucellosis, 599
Sodium chloride, deficiency; effect of sodium chloride deprivation on growing rat, 260
Solway, A. J. L.: Medioncrosis of aorta, 380
Specimens: See Tissue
Spine, arthritis; ankylosing (Strümpell-Marie) spondylitis, 303
Spleen, capsule, hepatic heterotopy in, 377
cysts, 550
cytologic changes in bronchogenic carcinoma following treatment with nitrogen mustard (methyl-bis β -chloroethyl amine), 503

- Spleen—Continued
 Hypertrophy: See Splenomegaly
 quantitative approach to study of splenomegaly, 320
- Splenomegaly: See also Anemia, splenic
 quantitative approach to study of, 320
- Spondylitis: See Spine, arthritis
- Stains and Staining; argentaffin cells of human appendix; comparative study of results obtained with modified Schmorl and Masson techniques, 83
 significance of beta granules in islets of Langerhans of pancreas, 159
 silvering of lepra bacilli in tissues, 542
- Stark, E.: Diffuse plasma cell myelomatosis, 183
- Sternum, Puncture: See Bones, marrow
- Steroids; cholesterol of human adrenal gland; its significance in relation to adrenal function and structure; 451
- Still's Disease: See Arthritis, rheumatoid
- Stowell, R. E.: Nucleic acids and cytologic changes in regenerating rat liver, 164
- Strümpell-Marlo Disease: See Spine, arthritis
- Succinylsulfathiazole: See Sulfonamides
- Sulfadiazine: See Sulfonamides
- Sulfonamides; control of hepatic coccidiosis of rabbits with succinylsulfathiazole U. S. P.; study of mode of action of sulfonamides, 128
- Suprarenal Preparations: See Adrenal Preparations
- Suprarenals: See Adrenals
- Sway-Back: See under Nervous System
- Tamaki, H. T.: Splenic cysts, 550
- Tedeschi, C. G.: Mammary lipoma, 386
- Teeth; postmortem examination of teeth and supporting structures to aid in personal identification, 119
- Teratoma; dyschondroplasia with hemangiomatosis (Maffucci's syndrome) and teratoid tumor of ovary, 74
 and adamantinoma, limitation of concept of, 256
- Terminology: See Nomenclature
- Testes, adult carcinomas of, 236
 interstitial cell tumor of, 230
 tumors; interstitial cell and miscellaneous neoplasms, 229
- Thorax: See Heart; Lungs; etc.
- Thüringer, J. M.: Plasma cell mastitis, 313
- Thymoma; comparison of thymic hyperplasia in myasthenia gravis and exophthalmic goiter, 212
- Thymus; comparison of thymic hyperplasia in myasthenia gravis and exophthalmic goiter, 212
- Thyroid, hyperthyroidism, 308
 hyperthyroidism; comparison of thymic hyperplasia in myasthenia gravis and exophthalmic goiter, 212
- Tissue: See also Cells
 cytologic changes in bronchogenic carcinoma following treatment with nitrogen mustard (methyl-bis β -chloroethyl amine), 503
 development of state refractory to growth of mouse tumor implanted in anterior chamber of guinea pig eye, 35
 genesis of gangrenous and reparative processes in trench foot, 1
 influence of local acidification of tissue bordering cancerous growths with reference to eosinophil, paneth cell and acidophilic plasma cell, 107
 significance of agonal changes in human liver, 132
 silvering of lepra bacilli in, 542
 spontaneous demyelinating diseases of animals; study in comparative pathology, 567
- Staining: See Stains and Staining
- Toxemia; interrelationship of diseases of liver and brain, 268
- Transplantation; development of state refractory to growth of mouse tumor implanted in anterior chamber of guinea pig eye, 35
- Trench Foot: See Foot, trench
- Tuberculosis: See also under names of various diseases, organs and regions
 sarcoidosis involving heart; report of case with sudden death, 289
- Tumors: See also Adamantinoma; Adenocarcinoma; Adenoma; Angioma; Arrhenoblastoma; Cancer; Lipoma; Myeloma; Sarcoma; Seminoma; Teratoma; and under names of organs and regions, as Breast; Eyes; Ovary; Parotid Gland; Pituitary Body; Testes; etc.
 metastatic, of testes, 241
 vascular and fibroblastic, of testes, 241
- Umiker, W.: Fatal viral hepatitis complicated by phlegmonous cecitis and ileocecal intussusception, 493
- Urinary Tract: See Kidneys
- Urology; Francis Amory Prize of American Academy of Arts and Sciences, 287
- Valsalva Sinus: See Aneurysm
- Van Leeuwen, M. J.: Postmortem examination of teeth and supporting structures to aid in personal identification, 119
- Vasa Vasorum: See Aorta
- Vasomotor System: See Blood pressure; Blood vessels; etc.
- Veins: See Blood vessels; Cardiovascular System; Embolism; etc.
 Pressure: See Blood pressure
- Vertebrae: See Spine
- Veterinary Medicine; spontaneous demyelinating diseases of animals; study in comparative pathology, 567
- Viscera; diffuse plasma cell myelomatosis, 183
 lesions, in case of rheumatoid arthritis, 59
- Vitamins: See also Diet and Dietetics; etc.
 B, deficiency; effects of folic acid deficiency and folic acid antagonist on chicks, 559
 B; effects of folic acid antagonists inoculated in embryonated eggs, 441
- Viruses: See also Hepatitis, Infectious; Polio-myelitis; etc.
 fatal viral hepatitis complicated by phlegmonous cecitis and ileocecal intussusception, 493
 spontaneous demyelinating diseases of animals; study in comparative pathology, 567
 viral versus toxic hepatic necrosis, 338
- Von Haam, E.: Hepatic heterotopy in splenic capsule, 377
- Wagley, P. F.: Effects of folic acid antagonists inoculated in embryonated eggs, 441
- War: See Military Medicine
- Ware, P. F.: Cytologic changes in bronchogenic carcinoma following treatment with nitrogen mustard (methyl-bis β -chloroethyl amine), 503
- Williams, R. H.: Cholesterol of human adrenal gland; its significance in relation to adrenal function and structure, 451
- Wilson's Disease: See Lenticular Nucleus, degeneration
- Woll, E.: Effects of folic acid deficiency and folic acid antagonist on chicks, 559
- Xanthomatosis: See Anemia, splenic
- Young, R. L.: Paradoxical embolism; review of literature, with report of case in which this condition followed administration of "dicumarol," 43

